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(71) Applicant *(for all designated States except US)*: ISIS PHARMACEUTICALS, INC. [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MONIA, Brett, P. [US/US]; 7605 Nueva Castilla Way, La Costa, CA 92009 (US). COWSERT, Lex, M. [US/US]; 3008 Newshire Street, Carlsbad, CA 92008 (US).

(74) Agents: LICATA, Jane, Massey et al.; Law Offices of Jane Massey Licata, 66 E. Main Street, Marlton, NJ 08053 (US).

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(54) Title: ANTISENSE MODULATION OF AKT-2 EXPRESSION

(57) Abstract

Antisense compounds, compositions and methods are provided for modulating the expression of Akt-2. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding Akt-2. Methods of using these compounds for modulation of Akt-2 expression and for treatment of diseases associated with expression of Akt-2 are provided.

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ANTISENSE MODULATION OF AKT-2 EXPRESSION

FIELD OF THE INVENTION

The present invention provides compositions and methods for modulating the expression of Akt-2. In particular, this invention relates to antisense compounds, particularly oligonucleotides, specifically hybridizable with nucleic acids encoding human Akt-2. Such oligonucleotides have been shown to modulate the expression of Akt-2.

BACKGROUND OF THE INVENTION

One of the principal mechanisms by which cellular regulation is effected is through the transduction of extracellular signals across the membrane that in turn modulate biochemical pathways within the cell. Protein phosphorylation represents one course by which intracellular signals are propagated from molecule to molecule resulting finally in a cellular response. These signal transduction cascades are highly regulated and often overlapping as evidenced by the existence of many protein kinases as well as phosphatases. Phosphorylation of proteins occurs predominantly at serine, threonine, or tyrosine residues and protein kinases have therefore been classified by their specificity of phosphorylation site i.e. serine/threonine kinases and tyrosine kinases. Because phosphorylation is such a ubiquitous process within cells and because cellular phenotypes are largely influenced by the activity of these pathways, it is currently believed that a number of disease states and/or disorders are a result of either aberrant activation or functional mutations in the molecular components of kinase cascades. Consequently, considerable attention has been devoted to the characterization of these proteins. Akt-2 (also known as PKB beta and RAC-PK beta) is a member of the AKT/PKB family of serine/threonine kinases and has been shown to be overexpressed in several types of cancers

as well as a mediator of normal cellular functions. Akt-2, like other members of the AKT/PKB family, is located in the cytosol of unstimulated cells and translocates to the nucleus following stimulation by several ligands including mitogens and survival factors (Meier et al., *J. Biol. Chem.*, **1997**, *272*, 30491-30497). Other studies have shown that this activation is through PI3 kinase which is wortmannin sensitive (Franke et al., *Science*, **1997**, *275*, 665-668). It is through the pleckstrin homology domain (PH) within the protein that Akt-2 binds to the lipid products of PI3 kinase allowing presentation of Akt-2 to its upstream activators by directing its translocation to the membrane. Phosphorylation of Akt-2 is necessary for its activation and the kinase responsible for this activation is PDK1 (Cohen et al., *FEBS Lett.*, **1997**, *410*, 3-10).

Once localized to the membrane, Akt-2 mediates several functions within the cell including the metabolic effects of insulin (Calera et al., *J. Biol. Chem.*, **1998**, *273*, 7201-7204), induction of differentiation and/or proliferation, protein synthesis and stress responses (Alessi and Cohen, *Curr. Opin. Genet. Dev.*, **1998**, *8*, 55-62). Manifestations of altered Akt-2 regulation appear in both injury and disease, the most important role being in the development of cancer. The first description of Akt-2 was in association with human ovarian carcinomas where the expression of the protein was found to be amplified in 15% of the cases (Cheng et al., *Proc. Natl. Acad. Sci. U S A*, **1992**, *89*, 9267-9271). It was also found to be overexpressed in 12% of pancreatic cancers (Cheng et al., *Proc. Natl. Acad. Sci. U S A*, **1996**, *93*, 3636-3641). In addition, Bellacosa et al. demonstrated Akt-2 overexpression in 12.1% of ovarian carcinomas and 2.8% of breast carcinomas, and that the amplification was

especially frequent in undifferentiated tumors (50%) suggesting that Akt-2 may be associated with tumor aggressiveness (Bellacosa et al., *Int. J. Cancer*, **1995**, *64*, 280-285).

Currently, there are no known therapeutic agents which effectively inhibit the synthesis of Akt-2. Consequently, there remains a long felt need for additional agents capable of effectively inhibiting Akt-2 function.
To date, strategies aimed at inhibiting Akt-2 function have involved the use of antibodies and transfection of antisense constructs of Akt-2 in tumor cell lines. Cheng et al. demonstrated in studies of tumor transplants using nude mice, that invasiveness and tumorigenicity were reduced in tumors transfected with antisense constructs expressing a 1.2 kb fragment of Akt-2 cDNA in the antisense orientation as compared to those cell lines not containing the Akt-2 antisense construct (Cheng et al., *Proc. Natl. Acad. Sci. U S A*, **1996**, *93*, 3636-3641).

Antisense oligonucleotides, therefore, provide a promising new pharmaceutical tool for the effective modification of the expression of specific genes including Akt-2.

SUMMARY OF THE INVENTION

The present invention is directed to antisense compounds, particularly oligonucleotides, which are targeted to a nucleic acid encoding Akt-2, and which modulate the expression of Akt-2. Pharmaceutical and other compositions comprising the antisense compounds of the invention are also provided. Further provided are methods of modulating the expression of Akt-2 in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention. Further provided are methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression

of Akt-2 by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention employs oligomeric antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding Akt-2, ultimately modulating the amount of Akt-2 produced. This is accomplished by providing antisense compounds which specifically hybridize with one or more nucleic acids encoding Akt-2. As used herein, the terms "target nucleic acid" and "nucleic acid encoding Akt-2" encompass DNA encoding Akt-2, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as "antisense". The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of Akt-2. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target.

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a

particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is a nucleic acid molecule encoding Akt-2. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the

codon or codons that are used in vivo to initiate translation of an mRNA molecule transcribed from a gene encoding Akt-2, regardless of the sequence(s) of such codons.

It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA

via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites, i.e., intron-exon junctions, may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide

and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway. Antisense modulation has, therefore, been harnessed for research use.

The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic

uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

While antisense oligonucleotides are a preferred form of antisense compound, the present invention comprehends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 30 nucleobases. Particularly preferred are antisense oligonucleotides comprising from about 8 to about 30 nucleobases (i.e. from about 8 to about 30 linked nucleosides). As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the

pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having

normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677;

5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*, **1991**, 254, 1497-1500.

Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-O-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester backbone is represented as $-O-P-O-CH_2-$] of the above referenced U.S. patent 5,489,677, and the amide backbones of the above referenced U.S. patent 5,602,240. Also preferred are oligonucleotides having

morpholino backbone structures of the above-referenced U.S. patent 5,034,506.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, **1995**, *78*, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow.

Other preferred modifications include 2'-methoxy (2'-O-CH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar

mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by

Englisch et al., *Angewandte Chemie*, International Edition, **1991**, *30*, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S.T. and Lebleu, B. , ed., CRC Press, **1993**. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, **1993**, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. 3,687,808, as well as U.S.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and United States patent 5,750,692, which is commonly owned with the instant application and also herein incorporated by reference.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but

are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Let.*, **1994**, *4*, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, **1992**, *660*, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.*, **1993**, *3*, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, **1992**, *20*, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, **1991**, *10*, 1111-1118; Kabanov et al., *FEBS Lett.*, **1990**, *259*, 327-330; Svinarchuk et al., *Biochimie*, **1993**, *75*, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, **1995**, *36*, 3651-3654; Shea et al., *Nucl. Acids Res.*, **1990**, *18*, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, **1995**, *14*, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, **1995**, *36*, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, **1995**, *1264*, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, **1996**, *277*, 923-937.

Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241,

5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary,

associated nucleic acid hybridization techniques known in the art.

Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

The antisense compounds of the invention are synthesized in vitro and do not include antisense compositions of biological origin, or genetic vector constructs designed to direct the in vivo synthesis of antisense molecules.

The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative

United States patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S.: 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE [(S-acetyl-2-thioethyl) phosphate] derivatives according to the methods disclosed in WO 93/24510 to Gosselin et al., published December 9, 1993 or in WO 94/26764 to Imbach et al.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," *J. of Pharma Sci.*, **1977**, *66*, 1-19). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a "pharmaceutical addition salt" includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid,

lactic acid, oxalic acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid. Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible.

For oligonucleotides, preferred examples of pharmaceutically acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid,

p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine.

The antisense compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of Akt-2 is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, e.g., to prevent or delay infection, inflammation or tumor formation, for example.

The antisense compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding Akt-2, enabling sandwich and other assays to easily be constructed to exploit this fact. Hybridization of the antisense oligonucleotides of the invention with a nucleic acid encoding Akt-2 can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of Akt-2 in a sample may also be prepared.

The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or

systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but

are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The preparation of such compositions and formulations is generally known to those skilled in the pharmaceutical and formulation arts and may be applied to the formulation of the compositions of the present invention.

Emulsions

The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, **1985**, p. 301). Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be either water-in-oil (w/o) or of the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and

water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., **1988**, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the

hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid,

carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of reasons of ease of formulation, efficacy from an absorption and bioavailability standpoint. (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245;

Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

In one embodiment of the present invention, the compositions of oligonucleotides and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, *in: Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., **1989**, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, **1985**, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355,

Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., *Pharmaceutical Research*, **1994**, *11*, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*, **1993**, *13*, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., *Pharmaceutical Research*, **1994**, *11*, 1385; Ho *et al.*, *J. Pharm. Sci.*, **1996**, *85*, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or oligonucleotides. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of oligonucleotides and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of oligonucleotides and nucleic acids

within the gastrointestinal tract, vagina, buccal cavity and other areas of administration.

Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the oligonucleotides and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories - surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al., Critical Reviews in Therapeutic Drug Carrier Systems,* **1991**, p. 92). Each of these classes has been discussed above.

Liposomes

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*.

In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each

with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes. As the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into

the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.*, *Biochem. Biophys. Res. Commun.*, ***1987,*** *147*, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, *Journal of Controlled Release*, **1992**, *19*, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (*e.g.* as a solution or as an emulsion) were ineffective (Weiner *et al.*, *Journal of Drug Targeting*, **1992**, *2*, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis *et al.*, *Antiviral Research*, **1992**, *18*, 259-265).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. *S.T.P.Pharma. Sci.*, **1994**, 4, 6, 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is

derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, *FEBS Letters*, **1987**, *223*, 42; Wu *et al.*, *Cancer Research*, **1993**, *53*, 3765). Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, **1987**, *507*, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, **1988**, *85*, 6949). U.S. Patent No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Patent No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-*sn*-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al.*).

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto *et al.* (*Bull. Chem. Soc. Jpn.*, **1980**, *53*, 2778) described liposomes comprising a nonionic detergent, $2C_{12}15G$, that contains a PEG moiety. Illum *et al.* (*FEBS Lett.*, **1984**, *167*, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols

(*e.g.*, PEG) are described by Sears (U.S. Patent Nos. 4,426,330 and 4,534,899). Klibanov *et al.* (*FEBS Lett.*, **1990**, *268*, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume *et al.* (*Biochimica et Biophysica Acta*, **1990**, *1029*, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle *et al.* (U.S. Patent Nos. 5,013,556 and 5,356,633) and Martin *et al.* (U.S. Patent No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Patent No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky *et al.*) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi *et al.*). U.S. Patent Nos. 5,540,935 (Miyazaki *et al.*) and 5,556,948 (Tagawa *et al.*) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry *et al.* discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Patent No. 5,264,221 to Tagawa *et al.* discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an antisense RNA. U.S. Patent No. 5,665,710 to Rahman *et al.* describes certain methods of encapsulating

oligodeoxynucleotides in liposomes. WO 97/04787 to Love *et al.* discloses liposomes comprising antisense oligonucleotides targeted to the raf gene.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, *e.g.* they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending

on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, 1988, p. 285).

Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligonucleotides through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi *et al.*, *J. Pharm. Pharmacol.*, **1988**, *40*, 252).

Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-*rac*-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-10} alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, p.92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1990**, 7, 1-33; El Hariri *et al.*, J. Pharm. Pharmacol., **1992**, 44, 651-654).

Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, **1996**, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate),

ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, page 92; Swinyard, Chapter 39 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, **1990**, pages 782-783; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1990**, *7*, 1-33; Yamamoto *et al.*, *J. Pharm. Exp. Ther.*, **1992**, *263*, 25; Yamashita *et al.*, *J. Pharm. Sci.*, **1990**, *79*, 579-583).

Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotides through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, **1993**, *618*, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), *N*-acyl derivatives of collagen, laureth-9 and *N*-amino acyl derivatives of beta-diketones (enamines)(Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1990**, 7, 1-33; Buur *et al.*, *J. Control Rel.*, **1990**, *14*, 43-51).

Non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that

nonetheless enhance absorption of oligonucleotides through the alimentary mucosa (Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1990**, *7*, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, *J. Pharm. Pharmacol.*, **1987**, *39*, 621-626).

Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Patent No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo *et al.*, PCT Application WO 97/30731), are also known to enhance the cellular uptake of oligonucleotides.

Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity *per se*) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier

compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate oligonucleotide in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, *Antisense Res. Dev.*, **1995**, *5*, 115-121; Takakura *et al.*, *Antisense & Nucl. Acid Drug Dev.*, **1996**, *6*, 177-183).

Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, *etc.*, when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch

glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc.*).

Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage

forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include, but are not limited to, anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 1206-1228). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in

compositions of the invention. See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., **1987**, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in in vitro and in vivo animal models. In general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy

to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES

Example 1

Nucleoside Phosphoramidites for Oligonucleotide Synthesis

Deoxy and 2'-alkoxy amidites

2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial sources (e.g. Chemgenes, Needham MA or Glen Research, Inc. Sterling VA). Other 2'-O-alkoxy substituted nucleoside amidites are prepared as described in U.S. Patent 5,506,351, herein incorporated by reference. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides was utilized, except the wait step after pulse delivery of tetrazole and base was increased to 360 seconds.

Oligonucleotides containing 5-methyl-2'-deoxycytidine (5-Me-C) nucleotides were synthesized according to published methods [Sanghvi, et. al., *Nucleic Acids Research*, **1993**, *21*, 3197-3203] using commercially available phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham MA).

2'-Fluoro amidites

2'-Fluorodeoxyadenosine amidites

2'-fluoro oligonucleotides were synthesized as described previously [Kawasaki, et. al., *J. Med. Chem.*, **1993**, *36*, 831-841] and United States patent 5,670,633,

herein incorporated by reference. Briefly, the protected nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine was synthesized utilizing commercially available 9-beta-D-arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-alpha-fluoro atom is introduced by a S_N2-displacement of a 2'-beta-trityl group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine was selectively protected in moderate yield as the 3',5'-ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and N6-benzoyl groups was accomplished using standard methodologies and standard methods were used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-phosphoramidite intermediates.

2'-Fluorodeoxyguanosine

The synthesis of 2'-deoxy-2'-fluoroguanosine was accomplished using tetraisopropyldisiloxanyl (TPDS) protected 9-beta-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyryl-arabinofuranosylguanosine. Deprotection of the TPDS group was followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation was followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies were used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

2'-Fluorouridine

Synthesis of 2'-deoxy-2'-fluorouridine was accomplished by the modification of a literature procedure in which 2,2'-anhydro-1-beta-D-arabinofuranosyluracil was treated with 70% hydrogen fluoride-pyridine. Standard procedures were used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

2'-Fluorodeoxycytidine

2'-deoxy-2'-fluorocytidine was synthesized via

amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give N4-benzoyl-2'-deoxy-2'-fluorocytidine. Standard procedures were used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

2'-O-(2-Methoxyethyl) modified amidites

2'-O-Methoxyethyl-substituted nucleoside amidites are prepared as follows, or alternatively, as per the methods of Martin, P., *Helvetica Chimica Acta*, **1995**, *78*, 486-504.

2,2'-Anhydro[1-(beta-D-arabinofuranosyl)-5-methyluridine]

5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum was dried in a vacuum oven (60°C at 1 mm Hg for 24 h) to give a solid that was crushed to a light tan powder (57 g, 85% crude yield). The NMR spectrum was consistent with the structure, contaminated with phenol as its sodium salt (ca. 5%). The material was used as is for further reactions (or it can be purified further by column chromatography using a gradient of methanol in ethyl acetate (10-25%) to give a white solid, mp 222-4°C).

2'-O-Methoxyethyl-5-methyluridine

2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol

(1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160°C. After heating for 48 hours at 155-160°C, the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH_3CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH_2Cl_2/acetone/MeOH (20:5:3) containing 0.5% Et_3NH. The residue was dissolved in CH_2Cl_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product. Additional material was obtained by reworking impure fractions.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH_3CN (200 mL). The residue was dissolved in $CHCl_3$ (1.5 L) and extracted with 2x500 mL of saturated $NaHCO_3$ and 2x500 mL of saturated NaCl. The organic phase was dried over Na_2SO_4, filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/hexane/acetone (5:5:1) containing 0.5% Et_3NH. The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total

yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by TLC by first quenching the TLC sample with the addition of MeOH. Upon completion of the reaction, as judged by TLC, MeOH (50 mL) was added and the mixture evaporated at 35°C. The residue was dissolved in $CHCl_3$ (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water layers were back extracted with 200 mL of $CHCl_3$. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/hexane(4:1). Pure product fractions were evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later fractions.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine

A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to -5°C and stirred for 0.5 h using an overhead stirrer. $POCl_3$ was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10°C, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the latter solution. The resulting reaction mixture was stored

overnight in a cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed with 1x300 mL of $NaHCO_3$ and 2x300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2x200 mL). The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH_3 gas was added and the vessel heated to 100°C for 2 hours (TLC showed complete conversion). The vessel contents were evaporated to dryness and the residue was dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyl-cytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, TLC showed the reaction to be approximately 95% complete. The solvent was evaporated and the residue azeotroped with MeOH (200 mL). The residue was dissolved in $CHCl_3$ (700 mL) and extracted with saturated $NaHCO_3$ (2x300 mL) and saturated NaCl (2x300 mL), dried over $MgSO_4$ and evaporated to give a residue (96

g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra-(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (TLC showed the reaction to be 95% complete). The reaction mixture was extracted with saturated $NaHCO_3$ (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over $MgSO_4$ and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column using EtOAc/hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites

2'-(Dimethylaminooxyethoxy) nucleoside amidites

2'-(Dimethylaminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(dimethylaminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and guanosine nucleoside amidites are prepared similarly to the thymidine (5-methyluridine) except the exocyclic amines are protected with a benzoyl moiety in the case of adenosine and cytidine and with isobutyryl in the case of guanosine.

5'-O-tert-Butyldiphenylsilyl-O²-2'-anhydro-5-methyluridine

O^2-2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese, Italy, 100.0g, 0.416 mmol), dimethylaminopyridine (0.66g, 0.013eq, 0.0054mmol) were dissolved in dry pyridine (500 ml) at ambient temperature under an argon atmosphere and with mechanical stirring. *tert*-Butyldiphenylchlorosilane (125.8g, 119.0mL, 1.1eq, 0.458mmol) was added in one portion. The reaction was stirred for 16 h at ambient temperature. TLC (Rf 0.22, ethyl acetate) indicated a complete reaction. The solution was concentrated under reduced pressure to a thick oil. This was partitioned between dichloromethane (1 L) and saturated sodium bicarbonate (2x1 L) and brine (1 L). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to a thick oil. The oil was dissolved in a 1:1 mixture of ethyl acetate and ethyl ether (600mL) and the solution was cooled to
-10°C. The resulting crystalline product was collected by filtration, washed with ethyl ether (3x200 mL) and dried (40°C, 1mm Hg, 24 h) to 149g (74.8%) of white solid. TLC and NMR were consistent with pure product.

5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine

In a 2 L stainless steel, unstirred pressure reactor was added borane in tetrahydrofuran (1.0 M, 2.0 eq, 622 mL). In the fume hood and with manual stirring, ethylene glycol (350 mL, excess) was added cautiously at first until the evolution of hydrogen gas subsided. 5'-O-tert-Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine (149 g, 0.311 mol) and sodium bicarbonate (0.074 g, 0.003 eq) were added with manual stirring. The reactor was sealed and heated in an oil bath until an internal temperature of 160 °C was reached and then maintained for 16 h (pressure < 100

psig). The reaction vessel was cooled to ambient and opened. TLC (Rf 0.67 for desired product and Rf 0.82 for ara-T side product, ethyl acetate) indicated about 70% conversion to the product. In order to avoid additional side product formation, the reaction was stopped, concentrated under reduced pressure (10 to 1mm Hg) in a warm water bath (40-100°C) with the more extreme conditions used to remove the ethylene glycol. [Alternatively, once the low boiling solvent is gone, the remaining solution can be partitioned between ethyl acetate and water. The product will be in the organic phase.] The residue was purified by column chromatography (2kg silica gel, ethyl acetate-hexanes gradient 1:1 to 4:1). The appropriate fractions were combined, stripped and dried to product as a white crisp foam (84g, 50%), contaminated starting material (17.4g) and pure reusable starting material 20g. The yield based on starting material less pure recovered starting material was 58%. TLC and NMR were consistent with 99% pure product.

2'-O-([2-phthalimidoxy)ethyl]-5'-*t*-butyldiphenylsilyl-5-methyluridine

5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine (20g, 36.98mmol) was mixed with triphenylphosphine (11.63g, 44.36mmol) and N-hydroxyphthalimide (7.24g, 44.36mmol). It was then dried over P_2O_5 under high vacuum for two days at 40°C. The reaction mixture was flushed with argon and dry THF (369.8mL, Aldrich, sure seal bottle) was added to get a clear solution. Diethyl-azodicarboxylate (6.98mL, 44.36mmol) was added dropwise to the reaction mixture. The rate of addition is maintained such that resulting deep red coloration is just discharged before adding the next drop. After the addition was complete, the reaction was stirred for 4 hrs. By that time TLC showed the completion of the

reaction (ethylacetate:hexane, 60:40). The solvent was evaporated in vacuum. Residue obtained was placed on a flash column and eluted with ethyl acetate:hexane (60:40), to get 2'-O-([2-phthalimidoxy)ethyl]-5'-*t*-butyldiphenylsilyl-5-methyluridine as white foam (21.819 g, 86%).

5'-O-*tert*-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine

2'-O-([2-phthalimidoxy)ethyl]-5'-*t*-butyldiphenylsilyl-5-methyluridine (3.1g, 4.5mmol) was dissolved in dry CH_2Cl_2 (4.5mL) and methylhydrazine (300mL, 4.64mmol) was added dropwise at -10°C to 0°C. After 1 h the mixture was filtered, the filtrate was washed with ice cold CH_2Cl_2 and the combined organic phase was washed with water, brine and dried over anhydrous Na_2SO_4. The solution was concentrated to get 2'-O-(aminooxyethyl) thymidine, which was then dissolved in MeOH (67.5mL). To this formaldehyde (20% aqueous solution, w/w, 1.1 eq.) was added and the resulting mixture was strirred for 1 h. Solvent was removed under vacuum; residue chromatographed to get 5'-O-*tert*-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy) ethyl]-5-methyluridine as white foam (1.95 g, 78%).

5'-O-*tert*-Butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine

5'-O-*tert*-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine (1.77g, 3.12mmol) was dissolved in a solution of 1M pyridinium p-toluenesulfonate (PPTS) in dry MeOH (30.6mL). Sodium cyanoborohydride (0.39g, 6.13mmol) was added to this solution at 10°C under inert atmosphere. The reaction mixture was stirred for 10 minutes at 10°C. After that the reaction vessel was removed from the ice bath and stirred at room temperature for 2 h, the reaction monitored by TLC (5% MeOH in CH_2Cl_2). Aqueous $NaHCO_3$ solution (5%, 10mL) was

added and extracted with ethyl acetate (2x20mL). Ethyl acetate phase was dried over anhydrous Na_2SO_4, evaporated to dryness. Residue was dissolved in a solution of 1M PPTS in MeOH (30.6mL). Formaldehyde (20% w/w, 30mL, 3.37mmol) was added and the reaction mixture was stirred at room temperature for 10 minutes. Reaction mixture cooled to 10°C in an ice bath, sodium cyanoborohydride (0.39g, 6.13mmol) was added and reaction mixture stirred at 10°C for 10 minutes. After 10 minutes, the reaction mixture was removed from the ice bath and stirred at room temperature for 2 hrs. To the reaction mixture 5% $NaHCO_3$ (25mL) solution was added and extracted with ethyl acetate (2x25mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated to dryness . The residue obtained was purified by flash column chromatography and eluted with 5% MeOH in CH_2Cl_2 to get 5'-O-*tert*-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine as a white foam (14.6g, 80%).

2'-O-(dimethylaminooxyethyl)-5-methyluridine

Triethylamine trihydrofluoride (3.91mL, 24.0mmol) was dissolved in dry THF and triethylamine (1.67mL, 12mmol, dry, kept over KOH). This mixture of triethylamine-2HF was then added to 5'-O-*tert*-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine (1.40g, 2.4mmol) and stirred at room temperature for 24 hrs. Reaction was monitored by TLC (5% MeOH in CH_2Cl_2). Solvent was removed under vacuum and the residue placed on a flash column and eluted with 10% MeOH in CH_2Cl_2 to get 2'-O-(dimethylaminooxyethyl)-5-methyluridine (766mg, 92.5%).

5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine

2'-O-(dimethylaminooxyethyl)-5-methyluridine (750mg, 2.17mmol) was dried over P_2O_5 under high vacuum overnight at 40°C. It was then co-evaporated with anhydrous pyridine (20mL). The residue obtained was dissolved in pyridine

(11mL) under argon atmosphere. 4-dimethylaminopyridine (26.5mg, 2.60mmol), 4,4'-dimethoxytrityl chloride (880mg, 2.60mmol) was added to the mixture and the reaction mixture was stirred at room temperature until all of the starting material disappeared. Pyridine was removed under vacuum and the residue chromatographed and eluted with 10% MeOH in CH_2Cl_2 (containing a few drops of pyridine) to get 5'-O-DMT-2'-O-(dimethylamino-oxyethyl)-5-methyluridine (1.13g, 80%).

5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine (1.08g, 1.67mmol) was co-evaporated with toluene (20mL). To the residue N,N-diisopropylamine tetrazonide (0.29g, 1.67mmol) was added and dried over P_2O_5 under high vacuum overnight at 40°C. Then the reaction mixture was dissolved in anhydrous acetonitrile (8.4mL) and 2-cyanoethyl-N,N,N^1,N^1-tetraisopropylphosphoramidite (2.12mL, 6.08mmol) was added. The reaction mixture was stirred at ambient temperature for 4 hrs under inert atmosphere. The progress of the reaction was monitored by TLC (hexane:ethyl acetate 1:1). The solvent was evaporated, then the residue was dissolved in ethyl acetate (70mL) and washed with 5% aqueous $NaHCO_3$ (40mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated. Residue obtained was chromatographed (ethyl acetate as eluent) to get 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] as a foam (1.04g, 74.9%).

2'-(Aminooxyethoxy) nucleoside amidites

2'-(Aminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(aminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs.

Adenosine, cytidine and thymidine nucleoside amidites are prepared similarly.

N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

The 2'-O-aminooxyethyl guanosine analog may be obtained by selective 2'-O-alkylation of diaminopurine riboside. Multigram quantities of diaminopurine riboside may be purchased from Schering AG (Berlin) to provide 2'-O-(2-ethylacetyl) diaminopurine riboside along with a minor amount of the 3'-O-isomer. 2'-O-(2-ethylacetyl) diaminopurine riboside may be resolved and converted to 2'-O-(2-ethylacetyl)guanosine by treatment with adenosine deaminase. (McGee, D. P. C., Cook, P. D., Guinosso, C. J., WO 94/02501 A1 940203.) Standard protection procedures should afford 2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine and 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine which may be reduced to provide 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine. As before the hydroxyl group may be displaced by N-hydroxyphthalimide via a Mitsunobu reaction, and the protected nucleoside may phosphitylated as usual to yield 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite].

Example 2

Oligonucleotide synthesis

Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

Phosphorothioates (P=S) are synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 h), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution. Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Example 3

Oligonucleoside Synthesis

Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

Example 4

PNA Synthesis

Peptide nucleic acids (PNAs) are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*, **1996**, *4*, 5-23. They may also be prepared in accordance with U.S. Patents 5,539,082, 5,700,922, and 5,719,262, herein incorporated by reference.

Example 5

Synthesis of Chimeric Oligonucleotides

Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type

wherein the "gap" segment of linked nucleosides is positioned between 5' and 3' "wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers".

[2'-O-Me]--[2'-deoxy]--[2'-O-Me] Chimeric Phosphorothioate Oligonucleotides

Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 380B, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle is modified by increasing the wait step after the delivery of tetrazole and base to 600 s repeated four times for RNA and twice for 2'-O-methyl. The fully protected oligonucleotide is cleaved from the support and the phosphate group is deprotected in 3:1 ammonia/ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hrs at room temperature is then done to deprotect all bases and sample was again lyophilized to dryness. The pellet is resuspended in 1M TBAF in THF for 24 hrs at room temperature to deprotect the 2' positions. The reaction is then quenched with 1M TEAA and the sample is then reduced to 1/2 volume by rotovac before being desalted on a G25 size exclusion column. The oligo recovered is then analyzed spectrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

[2'-O-(2-Methoxyethyl)]--[2'-deoxy]--[2'-O-(Methoxyethyl)] Chimeric Phosphorothioate Oligonucleotides

[2'-O-(2-methoxyethyl)]--[2'-deoxy]--[-2'-O-(methoxyethyl)] chimeric phosphorothioate oligonucleotides were prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

[2'-O-(2-Methoxyethyl)Phosphodiester]--[2'-deoxy Phosphorothioate]--[2'-O-(2-Methoxyethyl) Phosphodiester] Chimeric Oligonucleotides

[2'-O-(2-methoxyethyl phosphodiester]--[2'-deoxy phosphorothioate]--[2'-O-(methoxyethyl) phosphodiester] chimeric oligonucleotides are prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites, oxidization with iodine to generate the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to United States patent 5,623,065, herein incorporated by reference.

Example 6

Oligonucleotide Isolation

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides or oligonucleosides are purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes

ethanol. Synthesized oligonucleotides were analyzed by polyacrylamide gel electrophoresis on denaturing gels and judged to be at least 85% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in synthesis were periodically checked by ^{31}P nuclear magnetic resonance spectroscopy, and for some studies oligonucleotides were purified by HPLC, as described by Chiang et al., *J. Biol. Chem.* **1991**, *266*, 18162-18171. Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

Example 7

Oligonucleotide Synthesis - 96 Well Plate Format

Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a standard 96 well format. Phosphodiester internucleotide linkages were afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, CA, or Pharmacia, Piscataway, NJ). Non-standard nucleosides are synthesized as per known literature or patented methods. They are utilized as base protected beta-cyanoethyldiisopropyl phosphoramidites.

Oligonucleotides were cleaved from support and deprotected with concentrated NH_4OH at elevated temperature (55-60°C) for 12-16 hours and the released product then dried in vacuo. The dried product was then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

Example 8

Oligonucleotide Analysis - 96 Well Plate Format

The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96 well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy. All assay test plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the compounds on the plate were at least 85% full length.

Example 9

Cell culture and oligonucleotide treatment

The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. The following four cell types are provided for illustrative purposes, but other cell types can be routinely used.

T-24 cells:

The transitional cell bladder carcinoma cell line T-24 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). T-24 cells were routinely cultured in complete McCoy's 5A basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells were routinely passaged by trypsinization and

dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

A549 cells:

The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). A549 cells were routinely cultured in DMEM basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

NHDF cells:

Human neonatal dermal fibroblast (NHDF) were obtained from the Clonetics Corporation (Walkersville MD). NHDFs were routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville MD) supplemented as recommended by the supplier. Cells were maintained for up to 10 passages as recommended by the supplier.

HEK cells:

Human embryonic keratinocytes (HEK) were obtained from the Clonetics Corporation (Walkersville MD). HEKs were routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville MD) formulated as recommended by the supplier. Cells were routinely maintained for up to 10 passages as recommended by the supplier.

Treatment with antisense compounds:

When cells reached 80% confluency, they were treated with oligonucleotide. For cells grown in 96-well plates, wells were washed once with 200 µL OPTI-MEM™-1 reduced-serum medium (Gibco BRL) and then treated with 130 µL of OPTI-MEM™-1 containing 3.75 µg/mL LIPOFECTIN™ (Gibco BRL) and the desired oligonucleotide at a final concentration of 150 nM. After 4 hours of treatment, the medium was replaced with fresh medium. Cells were harvested 16 hours after oligonucleotide treatment.

Example 10

Analysis of oligonucleotide inhibition of Akt-2 expression

Antisense modulation of Akt-2 expression can be assayed in a variety of ways known in the art. For example, Akt-2 mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are taught in, for example, Ausubel, F.*M. et al., Current Protocols in Molecular Biology*, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc., **1993**. Northern blot analysis is routine in the art and is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., **1996**. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions. Other methods of PCR are also known in the art.

Akt-2 protein levels can be quantitated in a variety of ways well known in the art, such as immunoprecipitation,

Western blot analysis (immunoblotting), ELISA or fluorescence-activated cell sorting (FACS). Antibodies directed to Akt-2 can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional antibody generation methods. Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., **1997**. Preparation of monoclonal antibodies is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.4.1-11.11.5, John Wiley & Sons, Inc., **1997**.

Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.16.1-10.16.11, John Wiley & Sons, Inc., **1998**. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.8.1-10.8.21, John Wiley & Sons, Inc., **1997**. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.2.1-11.2.22, John Wiley & Sons, Inc., **1991**.

Example 11

Poly(A)+ mRNA isolation

Poly(A)+ mRNA was isolated according to Miura et al., *Clin. Chem.*, **1996**, *42*, 1758-1764. Other methods for poly(A)+ mRNA isolation are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.5.1-4.5.3, John Wiley & Sons, Inc., **1993**. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was

washed with 200 µL cold PBS. 60 µL lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) was added to each well, the plate was gently agitated and then incubated at room temperature for five minutes. 55 µL of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine CA). Plates were incubated for 60 minutes at room temperature, washed 3 times with 200 µL of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate was blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 µL of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C was added to each well, the plate was incubated on a 90°C hot plate for 5 minutes, and the eluate was then transferred to a fresh 96-well plate.

Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

Example 12

Total RNA Isolation

Total mRNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia CA) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 µL cold PBS. 100 µL Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 100 µL of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 15

seconds. 1 mL of Buffer RW1 was added to each well of the RNEASY 96™ plate and the vacuum again applied for 15 seconds. 1 mL of Buffer RPE was then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 15 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 10 minutes. The plate was then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 60 µL water into each well, incubating 1 minute, and then applying the vacuum for 30 seconds. The elution step was repeated with an additional 60 µL water.

Example 13

Real-time Quantitative PCR Analysis of Akt-2 mRNA Levels

Quantitation of Akt-2 mRNA levels was determined by real-time quantitative PCR using the ABI PRISM™ 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR, in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., JOE or FAM, obtained from either Operon Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either Operon Technologies Inc., Alameda, CA

or PE-Applied Biosystems, Foster City, CA) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular (six-second) intervals by laser optics built into the ABI PRISM™ 7700 Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

PCR reagents were obtained from PE-Applied Biosystems, Foster City, CA. RT-PCR reactions were carried out by adding 25 µL PCR cocktail (1x TAQMAN™ buffer A, 5.5 mM $MgCl_2$, 300 µM each of dATP, dCTP and dGTP, 600 µM of dUTP, 100 nM each of forward primer, reverse primer, and probe, 20 Units RNAse inhibitor, 1.25 Units AMPLITAQ GOLD™, and 12.5 Units MuLV reverse transcriptase) to 96 well plates containing 25 µL poly(A) mRNA solution. The RT reaction was carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the AMPLITAQ GOLD™, 40 cycles of a two-step PCR protocol were carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension). Akt-2 probes

and primers were designed to hybridize to the human Akt-2 sequence, using published sequence information (GenBank accession number M95936, incorporated herein as SEQ ID NO:1).

For Akt-2 the PCR primers were:
forward primer: AGCAGAATGCCAGCTGATGA (SEQ ID NO: 2)
reverse primer: GCAGGCAGCGTATGACAAAG (SEQ ID NO: 3) and the PCR probe was: FAM-ACCGAGAGGCCGCGACCCAA-TAMRA (SEQ ID NO: 4) where FAM (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

For GAPDH the PCR primers were:
forward primer: GAAGGTGAAGGTCGGAGTC (SEQ ID NO: 5)
reverse primer: GAAGATGGTGATGGGATTTC (SEQ ID NO: 6)and the PCR probe was: 5' JOE-CAAGCTTCCCGTTCTCAGCC- TAMRA 3' (SEQ ID NO: 7) where JOE (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

Example 14

Northern blot analysis of Akt-2 mRNA levels

Eighteen hours after antisense treatment, cell monolayers were washed twice with cold PBS and lysed in 1 mL RNAZOL™ (TEL-TEST "B" Inc., Friendswood, TX). Total RNA was prepared following manufacturer's recommended protocols. Twenty micrograms of total RNA was fractionated by electrophoresis through 1.2% agarose gels containing 1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc. Solon, OH). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by overnight capillary transfer using a Northern/Southern Transfer buffer system (TEL-TEST "B" Inc., Friendswood, TX). RNA transfer was confirmed by UV visualization. Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene,

Inc, La Jolla, CA).

Membranes were probed using QUICKHYB™ hybridization solution (Stratagene, La Jolla, CA) using manufacturer's recommendations for stringent conditions with a Akt-2 specific probe prepared by PCR using the forward primer AGCAGAATGCCAGCTGATGA (SEQ ID NO: 2) and the reverse primer GCAGGCAGCGTATGACAAAG (SEQ ID NO: 3). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, CA). Hybridized membranes were visualized and quantitated using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, CA). Data was normalized to GAPDH levels in untreated controls.

Example 15

Antisense inhibition of Akt-2 expression- phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of oligonucleotides were designed to target different regions of the human Akt-2 RNA, using published sequences (GenBank accession number M95936, incorporated herein as SEQ ID NO: 1). The oligonucleotides are shown in Table 1. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (Genbank accession no. M95936), to which the oligonucleotide binds. All compounds in Table 1 are oligodeoxynucleotides with phosphorothioate backbones (internucleoside linkages) throughout. The compounds were analyzed for effect on Akt-2 mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from three experiments. If present, "N.D." indicates "no data".

Table 1

Inhibition of Akt-2 mRNA levels by phosphorothioate oligodeoxynucleotides

ISIS#	REGION	TARGET SITE	SEQUENCE	% Inhibition	SEQ ID NO.
28960	5' UTR	1	tggacagggcacagtctc	70	8
28961	5' UTR	10	gaggcaccgtggacaggg	67	9
28962	Coding	89	tgacagacacctcattca	85	10
28963	Coding	95	ctttgatgacagacacct	77	11
28964	Coding	103	ccagccttctttgatgac	75	12
28965	Coding	110	tgtggagccagccttctt	72	13
28966	Coding	127	gatgtattcaccacgctt	N.D.	14
28967	Coding	132	gtcttgatgtattcacca	48	15
28968	Coding	180	ccaatgaaggagccgtcg	0	16
28969	Coding	242	ctacggagaagttgttta	58	17
28970	Coding	252	tggcattctgctacggag	82	18
28971	Coding	294	atgacaaaggtgttgggt	71	19
28972	Coding	373	ccgcatccactcctccct	88	20
28973	Coding	403	cttgaggctgttggcgac	82	21
28974	Coding	409	ccgctgcttgaggctgtt	71	22
28975	Coding	447	gagccacacttgtagtcc	89	23
28976	Coding	470	cagtcgtggaggagtcac	58	24
28977	Coding	481	ttccatctcctcagtcgt	81	25
28978	Coding	489	accgccacttccatctcc	N.D.	26
28979	Coding	498	gccttgctgaccgccact	82	27
28980	Coding	515	tggtcactttagcccgtg	0	28
28981	Coding	545	caaggagtttgagatagt	64	29
28982	Coding	576	accaggatgactttgcca	75	30
28983	Coding	636	atgatgacttcctttcgc	57	31
28984	Coding	658	gtgagcgacttcatcctt	75	32
28985	Coding	663	actgtgtgagcgacttca	62	33
28986	Coding	669	tcggtgactgtgtgagcg	66	34
28987	Coding	699	gggtgcctggtgttctgg	70	35
28988	Coding	761	actccatcacaaagcaca	80	36
28989	Coding	820	ctcctctgtgaagacacg	N.D.	37
28990	Coding	824	cccgctcctctgtgaaga	92	38
28991	Coding	864	tactcaagagccgagaca	N.D.	39
28992	Coding	980	cgtcactgatgccctctt	N.D.	40
28993	Coding	1276	ctccttggcatcgctggg	71	41
28994	Coding	1281	atgacctccttggcatcg	82	42
28995	Coding	1310	agttgatgctgaggaaga	49	43
28996	Coding	1414	gatggactgggcggtaaa	78	44
28997	Coding	1423	tgtgattgtgatggactg	70	45

28998	3' UTR	1543	cgtgcgtcctctgcgtgg	86	46
28999	3' UTR	1557	gtgatggcagcgagcgtg	62	47

As shown in Table 1, SEQ ID NOs 8, 9, 10, 11, 12, 13, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 31, 32, 33, 34, 35, 36, 38, 41, 42, 44, 45, 46 and 47 demonstrated at least 50% inhibition of Akt-2 expression in this assay and are therefore preferred.

Example 16:

Antisense inhibition of Akt-2 expression- phosphorothioate 2'-MOE gapmer oligonucleotides

In accordance with the present invention, a second series of oligonucleotides targeted to human Akt-2 were synthesized. The oligonucleotide sequences are shown in Table 2. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (Genbank accession no. M95936), to which the oligonucleotide binds.

All compounds in Table 2 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. Cytidine residues in the 2'-MOE wings are 5-methylcytidines.

Data were obtained by real-time quantitative PCR as described in other examples herein and are averaged from three experiments. If present, "N.D." indicates "no data".

Table 2

Inhibition of Akt-2 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS#	REGION	TARGET SITE	SEQUENCE	% Inhibition	SEQ ID NO.
29000	5' UTR	1	tggacagggcacagtctc	72	8
29001	5' UTR	10	gaggcaccgtggacaggg	0	9
29002	Coding	89	tgacagacacctcattca	65	10
29003	Coding	95	ctttgatgacagacacct	79	11
29004	Coding	103	ccagccttctttgatgac	73	12
29005	Coding	110	tgtggagccagccttctt	78	13
29006	Coding	127	gatgtattcaccacgctt	82	14
29007	Coding	132	gtcttgatgtattcacca	77	15
29008	Coding	180	ccaatgaaggagccgtcg	0	16
29009	Coding	242	ctacggagaagttgttta	67	17
29010	Coding	252	tggcattctgctacggag	0	18
29011	Coding	294	atgacaaaggtgttgggt	53	19
29012	Coding	373	ccgcatccactcctccct	81	20
29013	Coding	403	cttgaggctgttggcgac	100	21
29014	Coding	409	ccgctgcttgaggctgtt	92	22
29015	Coding	447	gagccacacttgtagtcc	78	23
29016	Coding	470	cagtcgtggaggagtcac	85	24
29017	Coding	481	ttccatctcctcagtcgt	20	25
29018	Coding	489	accgccacttccatctcc	48	26
29019	Coding	498	gccttgctgaccgccact	95	27
29020	Coding	515	tggtcactttagcccgtg	0	28
29021	Coding	545	caaggagtttgagatagt	76	29
29022	Coding	576	accaggatgactttgcca	85	30
29023	Coding	636	atgatgacttcctttcgc	0	31
29024	Coding	658	gtgagcgacttcatcctt	96	32
29025	Coding	663	actgtgtgagcgacttca	91	33
29026	Coding	669	tcggtgactgtgtgagcg	0	34
29027	Coding	699	gggtgcctggtgttctgg	0	35
29028	Coding	761	actccatcacaaagcaca	57	36
29029	Coding	820	ctcctctgtgaagacacg	100	37
29030	Coding	824	cccgctcctctgtgaaga	96	38
29031	Coding	864	tactcaagagccgagaca	100	39
29032	Coding	980	cgtcactgatgccctctt	90	40
29033	Coding	1276	ctccttggcatcgctggg	91	41
29034	Coding	1281	atgacctccttggcatcg	80	42
29035	Coding	1310	agttgatgctgaggaaga	80	43

29036	Coding	1414	gatggactgggcggtaaa	56	44
29037	Coding	1423	tgtgattgtgatggactg	74	45
29038	3' UTR	1543	cgtgcgtcctctgcgtgg	0	46
29039	3' UTR	1557	gtgatggcagcgagcgtg	88	47

As shown in Table 2, SEQ ID NOs 8, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 26, 27, 29, 30, 32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 47 demonstrated at least 40% inhibition of Akt-2 expression in this experiment and are therefore preferred.

Example 17

Western blot analysis of Akt-2 protein levels

Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 ul/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western blotting. Appropriate primary antibody directed to Akt-2 is used, with a radiolabelled or fluorescently labeled secondary antibody directed against the primary antibody species. Bands are visualized using a PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale CA).

What is claimed is:

1. An antisense compound 8 to 30 nucleotides in length targeted to a nucleic acid molecule encoding human Akt-2, wherein said antisense compound inhibits the expression of human Akt-2.

2. The antisense compound of claim 1 which is an antisense oligonucleotide.

3. The antisense compound of claim 2 wherein the antisense oligonucleotide has a sequence comprising SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47.

4. The antisense compound of claim 2 wherein the antisense oligonucleotide has a sequence comprising SEQ ID NO: 8, 10, 11, 12, 13, 17, 19, 20, 21, 22, 23, 24, 27, 29, 30, 32, 33, 36, 38, 41, 42, 44, 45 or 47.

5. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage.

6. The antisense compound of claim 5 wherein the modified internucleoside linkage is a phosphorothioate linkage.

7. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified sugar moiety.

8. The antisense compound of claim 7 wherein the modified sugar moiety is a 2'-O-methoxyethyl sugar moiety.

9. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified nucleobase.

10. The antisense compound of claim 9 wherein the modified nucleobase is a 5-methylcytosine.

11. The antisense compound of claim 2 wherein the antisense oligonucleotide is a chimeric oligonucleotide.

12. A composition comprising the antisense compound of claim 1 and a pharmaceutically acceptable carrier or diluent.

13. The composition of claim 12 further comprising a colloidal dispersion system.

14. The composition of claim 12 wherein the antisense compound is an antisense oligonucleotide.

15. A method of inhibiting the expression of Akt-2 in human cells or tissues comprising contacting said cells or tissues with the antisense compound of claim 1 so that expression of Akt-2 is inhibited.

16. A method of treating a human having a disease or condition associated with Akt-2 comprising administering to said animal a therapeutically or prophylactically effective amount of the antisense compound of claim 1 so that expression of Akt-2 is inhibited.

17. The method of claim 16 wherein the disease or condition is a hyperproliferative disorder.

18. The method of claim 17 wherein the hyperproliferative disorder is cancer.

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(71) Applicant *(for all designated States except US)*: VARIAGENICS, INC. [US/US]; One Kendall Square, Building 400, Cambridge, MA 02139–1562 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HOUSMAN, David [US/US]; 64 Homer Street, Newton, MA 02159 (US). LEDLEY, Fred, D. [US/US]; 433 Grove Street, Needham, MA 02192 (US). STANTON, Vincent, P., Jr. [US/US]; 32 Royal Road, Belmont, MA 02178 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon LLP, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071–2066 (US).

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(57) Abstract

This disclosure concerns genetic targets which have been found to be useful for allele specific anti–tumor therapy. The strategy for such therapy involves the steps of: (1) identification of alternative alleles of genes coding for proteins essential for cell viability or cell growth and the loss of one of these alleles in cancer cells due to loss of heterozygosity (LOH) and (2) the development of inhibitors with high specificity for the single remaining alternative allele of the essential gene retained by the tumor cell after LOH. Particular categories of appropriate target genes are described, along with specific exemplary genes within those categories and methods of using such target genes.

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DESCRIPTION

TARGET GENES FOR ALLELE-SPECIFIC DRUGS

BACKGROUND OF THE INVENTION

This invention is concerned with the field of treatment of proliferative disorders, including malignant and nonmalignant diseases, and with transplantation. Specifically, this invention is concerned with target genes for drugs that are useful for treating such diseases by providing allele-specific inhibition of essential cell functions.

The following information is provided to assist the understanding of the reader, none of that information is admitted to be prior art to the present invention.

The treatment of cancer is one of the most heavily investigated areas in biomedical research today. Although many anticancer drugs have been and continue to be discovered, there remains the immense problem of developing drugs that will be specifically toxic to cancer cells without killing normal cells and causing toxic, often permanent, damage to vital organs or even death. One common measure of the clinical usefulness of any anticancer drugs is its therapeutic index: the ratio of the median lethal dose (LD_{50}) to the median effective dose (ED_50) of the drug. With some cancer therapeutics this ratio is in the range of 4-6, or even 2-4, indicating a high risk of toxic side effects to the patient. Indeed, most anticancer drugs are associated with a high incidence of adverse drug events. The poor therapeutic index of most anticancer drugs not only limits the clinical efficacy of these drugs for the treatment of cancer, but limits their usefulness for treating many non-malignant, proliferative disorders.

A strategy for the development of anticancer agents having a high therapeutic

index is described in Housman, International Application PCT/US/94 08473 and Housman, INHIBITORS OF ALTERNATIVE ALLELES OF GENES ENCODING PROTEINS VITAL FOR CELL VIABILITY OR CELL GROWTH AS A BASIS FOR CANCER THERAPEUTIC AGENTS, U.S. Patent 5,702,890, issued December 30, 1997, which are hereby incorporated by reference in their entireties. As further described below, the method involves the identification of genes essential to cell growth or viability which are present in two or more allelic forms in normal somatic cells of a cancer patient and which undergo loss of heterozygosity in a cancer. Treatment of a cancer in an individual who is heterozygous with an allele specific inhibitor targeted to the single allele of an essential gene which is present in a cancer will inhibit the growth of the cancer cells. In contrast, the alternative allele present in non-cancerous cells (which have not undergone loss of heterozygosity) is able to express active product which supplies the essential gene function, so that the normal cells can survive and/or grow.

Cancer cells from an individual almost invariably undergo a loss of genetic material (DNA) when compared to normal cells. Frequently, this deletion of genetic material includes the loss of one of the two alleles of genes for which the normal somatic cells of the same individual are heterozygous, meaning that there are differences in the sequence of the gene on each of the parental chromosomes. The loss of one allele in the cancer cells is referred to as "loss of heterozygosity" (LOH). Recognizing that almost all, if not all, varieties of cancer undergo LOH, and that regions of DNA loss are often quite extensive, the genetic content of deleted regions in cancer cells was evaluated and it was found that genes essential for cell viability or cell growth are frequently deleted, reducing the cancer cell to only one copy. In this context, the term "deleted" refers to the loss of one of two copies of a chromosome or sub-chromosomal segment. Further investigation demonstrated that the loss of genetic material from cancer cells sometimes results

in the selective loss of one of two alleles of a certain essential gene at a particular locus or loci on a particular chromosome.

Based on this analysis, a therapeutic strategy for the treatment of cancer was developed, which will produce agents characterized by a high therapeutic index. The strategy includes: (1) identification of genes that are essential (or conditionally essential) for cell survival or growth; (2) identification of common alternative alleles of these genes; (3) identification of the absence of one of these alleles in cancer cells due to LOH and (4) development of specific inhibitors of the single remaining allele of the essential gene retained by the cancer cell, but not the alternative allele.

SUMMARY OF THE INVENTION

The utilization of inhibitors of alternative alleles, such as in the strategy described in Housman, *supra*, requires the provision of suitable target genes in order to identify such inhibitors and to implement corresponding diagnostic or therapeutic methods. Thus, as described below, the present invention identifies useful groups of genes which provide suitable target genes and further provides exemplary genes within those groups.

Additionally, the present inventors determined that LOH occurs not only in cancers, but also in non-cancerous proliferative disorders, though the location and frequency of LOH differs in different diseases, and established a method by which such non-cancerous proliferative disorders can be treated. Noncancer proliferative disorders include, for example, atherosclerotic plaques, premalignant metaplastic or dysplastic lesions, benign tumors, endometriosis, and polycystic kidney disease. In each disease, the administration of such an inhibitor would have cytotoxic or antiproliferative effects on the abnormally proliferating cells that exhibited LOH and contained only the sensitive allele of the target gene, but would not be toxic to

normal cells that contain also the alternative allele.

In addition, it was found that specific inhibitors of alternative alleles of an essential gene would be useful in managing transplantation in instances where the alleles in a donor bone marrow differ from the alleles in the recipient. For example, administration of an inhibitor of an allele that was present in a donor bone marrow but not the recipient could be used to treat graft-versus-host disease, suppressing proliferation of the donor marrow without toxicity to the recipient. Alternatively, an inhibitor of an allele that is present in the recipient but not the donor bone marrow could be used to enhance engraftment by preferentially creating space in the recipient bone marrow for the graft without inhibiting proliferation of the engrafted donor marrow.

In this context, a "gene" is a sequence of DNA present in a cell that directs the expression of a "biologically active" molecule or "gene product", most commonly by transcription to produce RNA ("RNA transcript") and translation to produce protein ("protein product"). Both RNA and protein may undergo secondary modifications such as those induced by reacting with other constituents of the cell which are also recognized as gene products. The gene product is most commonly a RNA molecule or protein, or a RNA or protein that is subsequently modified by reacting with, or combining with, other constituents of the cell. Such modifications may result, for example, in the modification of proteins to form glycoproteins, lipoproteins, and phosphoproteins, or other modifications known in the art. RNA may be modified by complexing with proteins, polyadenylation, or splicing. The term "gene product" refers to any product directly resulting from transcription of a gene. In particular this includes partial, precursor, and mature transcription products (*i.e.*, RNA), and translation products with or without further processing, such as lipidation, phosphorylation, glycosylation, or combinations of such processing (*i.e.*, polypeptides).

The term "target gene" refers to a gene where the gene, its RNA transcript, or its protein product are specifically inhibited or potentially inhibited by a drug. In references herein to genes or alleles, the term "encoding" refers to the entire gene sequence, including both coding and non-coding sequences unless clearly indicated otherwise.

The term "allele" refers to one specific form of a gene within a cell or within a population, the specific form differing from other forms of the same gene in the sequence of at least one, and frequently more than one, variant sites within the sequence of the gene. The sequences at these variant sites that differ between different alleles are termed "variances", "polymorphisms", or "mutations". The term "alternative allele", "alternative form", or "allelic form" refers to an allele that can be distinguished from other alleles by having distinct variances at at least one, and frequently more than one, variant site within the gene sequence.

It is recognized in the art that variances occur in the human genome at approximately one in every 100-500 bases. At most variant sites there are only two alternative variances, wherein the variances involve the substitution of one base for another or the insertion/deletion of a short gene sequence. Within a gene there may be several variant sites. Alternative alleles can be distinguished by the presence of alternative variances at a single variant site, or a combination of several different variances at different sites. In this invention, inhibitors targeted to a specific allelic form or subset of the allelic forms of a gene can be targeted to a specific variance in a selected variant site, or to an allele comprised of a set of variances at different sites. In most but not all cases, the target specificity is based on a nucleotide or amino acid change at a single variance site.

The term "proliferative disorder" refers to various cancers and disorders characterized by abnormal growth of somatic cells leading to an abnormal mass of

tissue which exhibits abnormal proliferation, and consequently, the growth of which exceeds and is uncoordinated with that of the normal tissues. The abnormal mass of cells is referred to as a "tumor", where the term tumor can include both localized cell masses and dispersed cells, The term "cancer" refers to a neoplastic growth and is synonymous with the terms "malignancy", or "malignant tumor". The treatment of cancers and the identification of anticancer agents is the concern of particularly preferred embodiments of the aspects of the present invention. Other abnormal proliferative diseases include "nonmalignant tumors", and "dysplastic" conditions including, but not limited to, leiomyomas, endometriosis, benign prostate hypertrophy, atherosclerotic plagues, and dysplastic epithelium of lung, breast, cervix, or other tissues. Drugs used in treating cancer and other non-cancer proliferative disorders commonly aim to inhibit the proliferation of cells and are commonly referred to as antiproliferative agents.

"Loss of heterozygosity", "LOH", or "allele loss" refers to the loss of one of the alleles of a gene from a cell or cell lineage previously having two alleles of that gene. Normal cells contain two copies of each gene, one inherited from each parent. When these two genes differ in their gene sequence, the cell is said to be "heterozygous". The term heterozygous indicates that a cell contains two different allelic forms of a particular gene and thus indicates that the allelic forms differ at at least one sequence variance site. When one allele is lost in a cell, that cell and its progeny cells, comprising its cell lineage, become "hemizygous" for that gene or "partially hemizygous" for a set of genes, and heterozygosity is lost. LOH occurs in all cancers and is a common characteristic of non-malignant, proliferative disorders. In general, many different genes will be affected by loss of heterozygosity in a cell which undergoes loss of heterozygosity. In many cancers 10-40% of all of the genes in the human genome (there are estimated to be 60,000-100,000 different genes in the genome) will exhibit LOH. In the context of this invention, these terms refer preferably to loss of heterozygosity of a gene

which has a particular sequence variance in normal somatic cells of an individual such that there is loss of heterozygosity with respect to that particular sequence variance. Also preferably, these terms refer to loss of heterozygosity of a particular sequence variance that is recognized by an inhibitor that will inhibit one allele of the gene present in normal cells of the individual, but not an alternative allele.

Preferably, loss of heterozygosity occurs before clonal or oligoclonal expansion of cells associated with a condition or disease, for example, cancer or non-cancer proliferative disorder. Cancer is a "clonal" disorder, meaning that all of the cells in the cancer or tumor are the progeny, or lineage, of a single cell which undergoes malignant transformation. Since cancer is clonal, any loss of heterozygosity or allele loss that occurs during the process of malignant transformation will be uniformly present throughout the lineage of the initial transformed cell. This results in the cancer cells uniformly and consistently having only one allelic form of the gene which is present in two allelic forms in normal cells.

Some of the non-malignant proliferative conditions that exhibit LOH are "oligoclonal", meaning that unlike cancers and most benign tumors, there are multiple, independently arising clonal populations, with discrete LOH events in each of the individual clones. The alleles subject to LOH may vary from one clone to another. Therefore treatment of these conditions preferably utilizes inhibitors of at least two allelic forms. Thus, methods relating to such disorders can utilize alternative alleles of one gene and/or allelic forms of additional genes. Certain noncancer, proliferative disorders are considered to be precursors for cancer. Such disorders progressively exhibit LOH until a single cell within the lesion caused by abnormal proliferation undergoes transformation and clonal expansion to form a cancer. Because LOH occurs in the precancerous condition,

the present invention provides a method for preventing cancer by administering drugs that are selectively toxic to cells in which LOH involving a gene that is essential for cell survival or proliferation creates a genetic difference between cancer cells and normal cells. Since certain cancers are predictably associated with a high frequency of LOH in certain locations, for example segments of chromosomes 7,8,10,11,13,16, and 18 in prostate cancer, administration of an allele-specific drug that inhibits one allele that is within such a region, in a patient who is heterozygous for alternative forms of the gene, would kill cells that undergo LOH before cancer occurs. Preferably, in the context of this invention, LOH refers to loss of an allelic form of an essential gene in cells that are involved in cancer or noncancer proliferative disorders, which has sequence variants in a population of interest, in an individual whose normal somatic cells are heterozygous for sequence variants of that gene.

As pointed out above, an important aspect of methods for treating cancer or noncancer proliferative disorders utilizing LOH of essential genes is the identification of suitable essential genes for use as target genes. In accord with that requirement, this invention identifies certain useful groups or categories of essential genes, and provides, as examples, specific genes within those categories which are found to be suitable as targets for allele specific inhibitors, in particular for killing cancer cells or reducing the proliferation of cells in cancer or noncancer proliferative disorders. Thus, the present invention provides suitable target genes and methods of utilizing those genes in allele specific or variance specific targeting. Such targets are essential genes, which can include conditionally essential genes. As further described below, suitable target genes include those essential genes which encode gene products necessary for maintaining the level of a cellular constituent within the levels required for cell survival or proliferation, or which encode a gene product required for cell proliferation. If the level of activity of an essential gene product is reduced, the level of the corresponding cellular

constituent will not be properly maintained or the cell will be unable to perform the cellular functions required for cell proliferation. Confirmation that such a gene undergoes LOH in a neoplastic condition, *e.g.*, a cancer, and that there are at least two alleles of the gene in the population that differ in one or more variant positions, indicates that the gene is a useful potential target gene in this invention for the identification of allele specific inhibitors and in other aspects of the invention.

Certain useful groups of target genes are described in which the essential genes have been grouped according to the type of essential cellular function in which the gene products are involved. Thus, the gene product of each of the individual genes within each of the categories or subcategories is itself essential to the cell. In particular, the categories of genes, or cell functions shown in Table 1(in the Detailed Description below) provide appropriate target genes. Particular exemplary target genes are also identified in Tables 1 and 2 and the Examples (including a GenBank accession number (or other sequence identifier as recognized by those skilled in the art) identifying the gene and providing a known sequence) which can be used for identifying allele specific inhibitors and for use in other aspects of this invention. Preferably the gene has the LOH frequency and at least one sequence variance in the gene has a heterozygosity rate in a population as indicated as preferable below, and occurs at only a single locus in the human genome.

An "essential" gene or gene product is one which is crucial to cell growth or viability. The terms "essential", "vital for cell viability or growth", or "essential for cell survival and proliferation" have the same meaning. A gene is essential if inhibition of the function of such a gene or gene product will kill the cell or inhibit its growth as determined by methods known in the art. Growth inhibition can be monitored as a reduction or preferably a cessation of cell proliferation.

Essentially can be demonstrated in a variety of different ways known in the art. Examples include, among others, generation of growth conditional mutants and identification of the affected genes, replacement of active genes with inactive mutants, cell fusion gene complementation analysis (see, *e.g.*, John Wasmuth, "Chinese Hamster Cell Protein Synthesis Mutants", Ch. 14 in Molecular Cell Genetics, Michael Gottesman, ed. Wiley, New York, 1985), and insertion of genetic suppressor elements leading to growth arrest (Pestov & Lau, 1994, *Proc. Natl. Acad. Sci. USA* 91:12549-12533). Other ways include the identification of conditionally lethal mutants, *e.g.*, temperature sensitive mutants and determination of the affected gene, genetic disruption of the gene by homologous recombination or other methods in organisms ranging from yeast to mice, inhibition of the gene by antisense oligonucleotides or ribozymes, and identification of the target of known cytotoxic drugs and other inhibitors. As further discussed below, the essentiality of a gene can depend on the conditions to which the cell is exposed. Thus, unless otherwise indicated, the term "essential gene" includes both "generally essential genes" and "conditionally essential genes". "Generally essential genes" are those which are strictly essential for cell survival or growth, or which are essential under the conditions to which the cell is normally exposed. Typically such conditions are the normal *in vivo* conditions or *in vitro* conditions which approximately replicate those *in vivo* conditions. Thus, in the methods described here utilizing essential genes, the method is carried out in conditions such that the gene product is required.

In connection with the determination of gene essentiality, it is generally recognized that the demonstration of essentiality of a gene in one organism is strongly suggestive that the homologous gene will be essential in another organism. This is especially true for genes which have relatively high levels of sequence conservation across a broad range of organisms. Thus, the identification of essential genes in prokaryotes or in lower eukaryotes such as yeast is indicative of

the identification of corresponding homologous essential genes or gene classes in higher eukaryotes such as humans. Therefore, studies of essential genes for non-human organisms provides useful information on likely human essential genes; an example is the Stanford *Saccharomyces cerevisiae* Database: http://genome-WWW Stanford.edu/cgr-bin/dbrun/SacchDB which provides a catalog of essential genes in yeast. It should be recognized, however, that not all essential genes from lower organisms will have recognized homologues in humans. It should also be recognized that the essential genes for a particular organism will generally not be restricted to those for which homology can be shown to essential genes in other organisms. Thus, genes may be essential in humans that are not essential in lower organisms.

In addition to generally essential genes, it is also recognized in the art that environmental factors can cause certain genes to be essential that are not essential under other conditions (including usual culture conditions). For example, certain genes involved in intermediary metabolism are not essential if the cell or organism is supplemented with high concentrations of a particular nutrient or chemical entity, but if that nutrient or chemical entity is absent or present at low levels, the gene product is essential. In another example, the administration of a drug that inhibits one or more functions within the cell can cause other functions to be essential that are not essential in the absence of the drug. In another example, subjecting a cell to harsh physical agents, such as radiation, can cause certain genes to be essential that are not essential under normal conditions. Such genes are essential under certain conditions associated with the therapy of cancer. The demonstration that such genes are present in the population in more than one allelic form and are subjected to loss of heterozygosity in cancer or noncancer proliferative disorders makes such genes targets for allele specific drugs for the treatment of such disorders.

Thus, a gene is said to be "conditionally essential" if it is essential for cell survival or proliferation in a specific environmental condition caused by the presence or absence of specific environmental constituents, pharmaceutical agents, including small molecules or biologicals, or physical factors such as radiation.

The term "cellular constituent" refers to chemical entities that comprise the substance of a living cell. In preferred embodiments, the cellular constituent is a protein or modified protein. Also, in preferred embodiments, the cellular constituent is an inorganic ion, an organic compound such as a lipid, carbohydrate, amino acid, organic acid, nucleoside, DNA, or RNA, or modified form of the preceding formed by the reaction of two constituents of the cell. In another embodiment, the constituent may comprise a structural element of the cell such as a membrane or cytoskeleton. In the preferred embodiment of this invention, cellular constituent refers to chemical entities, including compounds but also including simple ions, which are required for survival or proliferation of a human cell.

Certain cellular constituents of a cell are synthesized by the cell while others are not synthesized by the cell but are taken into the cell from its environment. Within the cell, constituents engage in various reactions to form new constituents by intermediary metabolism, are modified to form new constituents, and are preferentially compartmentalized in particular structures within the cell including, but not limited to, the nucleus, mitochondria, cytoplasm, or vesicles. Certain constituents are also specifically eliminated by the cell, or specific compartments within the cell, by degradation or excretion. In connection with cellular constituents, the term "maintaining the level" refers to maintaining the amount of the chemical entity normally associated with a specific cellular compartment or compartments and involves the action of various cellular processes, including synthesis, production, compartmentalization, transport, modification, combining

of two or more constituents, polymerization, elimination, degradation, and excretion. It is recognized in the art that the failure to maintain the level of certain cellular constituents within normal levels results in cell death, for example, cell death may result from inappropriate levels of proteins, DNA, or RNA, inappropriate levels of inorganic ions, inappropriate levels of organic compounds required for energy or other metabolic processes, or inappropriate intracellular structure. These examples are meant to be illustrative of the understanding of the meaning of the terms to those skilled in the art and not limiting.

In addition to the useful functional groups of essential genes described above, the present invention also provides useful groups of essential genes which are advantageous for allele specific targeting due to the genes undergoing LOH at certain frequencies in a disorder or other conditions and/or by having at least two allelic forms of the gene which appear in the population at particularly useful frequencies.

Thus, it is found that essential genes which undergo LOH in at least 10% of cases of a human cancer, and which exist in at least two allelic forms in a human population are advantageous targets. Preferably, the gene undergoes LOH in at least 20% of cases of a disorder, more preferably in at least 30%, still more preferably in at least 40%, and most preferably in at least 50% of such cases.

The LOH frequencies for a large number of different genetic markers for particular proliferative disorders are known in the art, and are used as indicators of the LOH frequency for neighboring essential genes. A number of LOH markers are provided in Fig. 3 (Loss of Heterozygosity Table). In one aspect of this invention, those essential genes which are located within about 20 megabases, more preferably within about 10 megabases, and most preferably within about 5 megabases of an identified marker or tumor suppressor gene which undergoes

LOH in at least 10, 20, 30, 40, or 50% of cases of a proliferative disorder, are particularly useful as they will undergo LOH at similar frequencies as the marker gene.

The relative locations of a marker and an essential gene can also be described by genetic, rather than physical, map distances, therefore, in preferred embodiments, an essential gene of this invention is preferably within about 20 centimorgans, more preferably within about 15 centimorgans, still more preferably within about 10 centimorgans, and most preferably within about 5 centimorgans of such an LOH marker or tumor suppressor gene. In preferred embodiments, the target gene is located near a reported marker which undergoes LOH at a frequency of at least 10, 20, 30, 40, or 50% for a proliferative disorder. A number of such markers and the associated chromosomal locations are provided in Fig. 3. Even more preferably, essential genes which map to a locus bracketed by two such markers are appropriate potential target genes, as the essential gene very probably will also undergo LOH at similar high frequencies. Preferably both markers undergo LOH at frequencies of at least 10, 20, 30, 40, or 50% of cases of a cancer. Thus, confirmation that an essential gene, for example, a gene from one of the functional groups described above, or one of the particular exemplary genes, maps close to a marker as just described, indicates that the gene is an appropriate potential target. Identification of one or more sequence variances in that gene and/or in the corresponding gene products allows screening or design of such inhibitors for potential treatment.

A useful way to determine the frequency of loss of heterozygosity for a tumor cell based on the physical position of the gene on chromosomes within the human genome has been described by Vogelstein et al., 1989, *Science* 244:207-211. These authors describe a measure of allele loss termed Fractional Allele Loss (FAL) which quantifies the extent of LOH in cancer based on LOH determinations

over each informative chromosomal arm. FAL is determined by dividing the number of informative chromosomal arms which undergo LOH by the total number of informative chromosomal arms, *i.e.*, each chromosome/arm with at least one heterozygous locus in normal cells. Examples of such FAL determinations are provided by Vogelstein et al., 1989 (FAL= 0.20 in colon cancer), and Cliby et al., 1993, *Cancer Research* 53:2393-2398 (FAL= 0.17 for low grade ovarian cancers, 0.40 for high grade ovarian cancers, 0.35 for all ovarian cancers).

These data indicate that genes on the chromosomal segment or chromosomal arm that is commonly lost in a cancer or non-cancer proliferative disorder are potential target genes. In preferred embodiments, the target gene is located on a chromosomal arm which is reported in the art or shown herein to contain a locus or loci which undergoes LOH at a frequency of at least 15, preferably at least 20%, still more preferably at least 25%, and most preferably at least 30, 40, or 50% in a proliferative disorder. As noted above, the frequency of LOH for a chromosomal arm is often utilized in calculating an average fraction of allele loss (FAL). Thus, a high LOH frequency for an arm or portion of an arm indicates that particular genes in the relevant chromosomal region will also undergo LOH at a comparable frequency, and thus define useful target genes. Preferably the target genes are those which are located on particular chromosomal arms which commonly undergo tumor-related LOH. In particular, these human chromosomal arms include 1p, 1q, 3p, 5q, 6p, 6q, 7q, 8p, 9p, 9q, 10q, 11p, 11q, 13q, 16q, 17p, 17q, 18p, 18q, and 22q. It is recognized that the LOH frequency is not uniform for all positions along an arm of a particular chromosome, however such LOH frequencies provide a strong indicator for LOH frequency at a potential target gene. Thus, mapping of an essential gene to these chromosomal arms or to high frequency LOH regions on these arms indicates that the gene is a potential target. Confirmation of the LOH of the particular gene and of the presence of at

least one sequence variance, and therefore of individuals heterozygous for such variances, indicates that the gene can be used for the identification of inhibitors targeting allelic forms of the gene which have a particular variance or variances and in the other aspects of this invention.

The term "high frequency LOH chromosomal region" refers to a chromosomal region which undergoes LOH at a frequency as indicated above, and include high frequency LOH chromosomal arms (at least 15% FAL), regions within the genetic or physical map distances indicated above of a chromosomal marker or tumor suppressor gene which undergoes LOH at a frequency as indicated above (at least 10%).

In connection with the location of a potential target gene with respect to a marker or tumor suppressor gene, the term "proximity" means that the target gene is located within a genetic or physical map distance of the reference gene or marker as stated above.

The present invention is aimed, in part, at treating cancer or proliferative disorders of any type in which LOH of an essential gene occurs at a frequency as indicated above. For example, this includes but is not limited to cancers and noncancer proliferative disorders provided in Tables 2 and 3 and Figure 3, or otherwise described herein. Table 2 and Fig. 3 describe a number of cancers for which LOH at substantial frequencies has been described in the art. Therefore, identification of an essential gene which maps to the LOH regions for a particular proliferative disorder, as described by genetic or physical mapping or by residence on a chromosomal arm or smaller region of an arm which is shown to undergo LOH, at high frequency in a proliferative disorder, identifies a potential target gene. Identification of sequence variances in that gene, such that normal somatic cells of individuals in a population are heterozygous for a variance and thus

contain two different alleles, confirms that the gene is a potential target. The target gene, its RNA transcript or protein product can then be used as targets for allele-specific inhibitors for treating the proliferative disorder or other uses as described in the aspects of this invention.

A further indication of useful target genes is provided by tumor-specific LOH of essential genes associated with tumor suppressor genes. LOH in certain cancers or noncancer proliferative disorders is frequently associated with specific chromosomal arms. This association is believed to be due, in many cases, to the presence of tumor suppressor genes located on those particular chromosomal arms, the loss of which eliminates the tumor suppressor function and contributes to the transformation of the cell. Consequently, essential genes which map near such a tumor suppressor gene are potential target genes for this invention. Preferably, the essential gene maps within a physical or genetic map distance as described above for LOH markers. As for the above categorization aspect, the LOH for a particular gene preferably is at least 10, 20, 30, 40, or 50% for a tumor, such as the cancers and types of cancers identified in Tables 2 and 3 and in Fig. 3. It should be noted that tumor suppressor genes themselves are rarely essential for cell survival or proliferation and not likely to be preferred targets for this invention.

Another group of essential genes which are potentially useful as target genes are those which are present in the population in at least two alternative forms or alleles containing one or more sequence variations, where the alternate forms occur at frequencies such that at least 10% of a population is heterozygous (*i.e.*, have two alternative forms of the gene), preferably so that at least 20%, more preferably at least 30%, and most preferably at least 40% are heterozygous. The term "heterozygote frequency" refers to the fraction of individuals in a population who have two alternative forms of a gene, or particular variances within a gene, in

their normal, somatic cells and are therefore heterozygous.

The term "allele frequency" refers to the fraction (or frequency of occurrence) of a specific allele as compared to all alleles in a population. It is recognized in the art that the heterozygote frequency and allele frequency are related and, for certain alleles, can be described by Hardy Weinberg equilibrium calculations. It will also be recognized that sequence variances that occur at high frequency in the population are commonly not deleterious to the health of the individuals who carry these genes and are commonly not disease genes or mutations that are associated with disease.

Methods for determining the heterozygote frequency or allele frequency or determining the number of individuals who are heterozygous for specific variances are known in the art, including but not limited to methods such as restriction fragment length polymorphism, hybridization of sequence specific nucleic acid probes to DNA or RNA sequences which include a sequence variance site, DNA sequencing, or mass spectrometry of amplified sequence fragments containing a sequence variance site. Methods that are useful for the discovery of genetic variances can also be used including, but not limited to, methods such as methods such as the SSCP technique (see Example 28), Enzymatic Mutation Detection technique (see Example 29), Denaturing Gradient Gel Electrophoresis, or sequencing. Identification of such genes which have sequence variances that are common in the general population and for which 10%, 20%, 30%, or 50% of the population are heterozygous for that gene provides genes which are particularly likely to be useful target genes for allele specific inhibition in this invention. Confirmation that the gene undergoes LOH at a useful frequency in a proliferative disorder, preferably in at least 10, 20, 30, 40, or 50% of cases of such a disorder indicates that the gene is useful as a potential target for identifying allele specific inhibitors for the treatment of proliferative disorders and in other aspects of this

invention.

Exemplary genes described herein are shown to contain numerous sequence variances which are present in human populations. While some sequence variances and alleles are common throughout diverse human populations, it is recognized in the art that the allele frequency of different genes will vary in different populations. For example, allele frequencies have been shown to differ between populations comprised of individuals of different races, populations comprised of individuals from different countries, populations comprised of individuals from different regions, populations comprised of individuals with common ethnic background, and even populations comprised of individuals from different religions. Alleles that are common in one population, may be rare in another. While the allele frequency of any particular gene may vary in different populations, the genes that are described below are those that occur such that at least 1% or 5% of a population is heterozygous for the sequence variance, preferably so that at least 10% or 20%, more preferably at least 30%, and most preferably at least 40% are heterozygous in a specific population that may be treated with inhibitors to treat cancer or other proliferative disorder in that population. Once a specific variance is identified in a certain gene, the allele frequency in any specific population can be easily determined using methods known in the art including the use of allele-specific hybridization probes, sequencing, or specific PCR reactions.

In this regard, "population" refers to a geographically, ethnically, or culturally defined group of individuals, or a group of individuals with a particular disease or a group of individuals that have proliferative diseases that may be treated by the present invention. Thus, in most cases a population will preferably encompass at least ten thousand, one hundred thousand, one million, ten million, or more individuals, with the larger numbers being more preferable. In special

circumstances, diseases will occur with high frequency in specific geographical regions or within specific familial, racial, or cultural groups, and a relevant population may usefully be considered to be a smaller group.

In the context of this invention, an alternative allele, or other reference to an appropriate target for the inhibitors of this invention refers to a form of a gene which differs in base sequence from at least one other allele or allelic form of the same gene. Usually, though not necessarily, the allelic forms of a gene will differ by, at most, several bases and may have only a single base difference (*i.e.*, a single sequence variance). The allelic forms, however, are ones which contain at least one sequence variance which appears in somatic cells of a population at an appreciable frequency, such that preferably at least 1%, more preferably at least 5%, still more preferably at least 10%, and most preferably at least 20% of the population are heterozygous for that specific sequence variance. This advantageously allows the convenient identification of potential patients, because an appreciable fraction of the population, and therefore also of the cancer patients will be heterozygous for sequence variances of the specific gene. In the context of this invention, different alleles need not result in different observable phenotypes under normal conditions. Preferably, a particular sequence variance produces no phenotypic effect on the physical condition of an individual having that variance until the variance is targeted by an allele specific inhibitor.

In connection with allele specific inhibitors and the methods of this invention, the terms "allelic form" or "alternative form of the target gene" or "sequence variance within the target gene" refer to either or both of the gene or a product of that gene including the RNA transcript or protein product. Thus, a particular inhibitor may act in an allele specific manner (which will often be variance specific) at any of those levels and preferably the inhibitor is targeted to a particular sequence variance of the specific allelic form.

As indicated above, two different allelic forms of a gene will have at least a one nucleotide difference in the nucleotide sequence of the gene. The difference can be of a variety of different types, including base substitution, single nucleotide insertion or deletion, multiple nucleotide insertion or deletion, and combinations of such differences. Thus, two allelic forms are sequence variants and will have at least one sequence variance, which refers to the sequence difference, between the allelic forms. However, there may also be more than one sequence variance between two allelic forms. The location of a sequence variance in a gene sequence is a "sequence variance site." This description applies to both the DNA and RNA sequences, and similarly applies to a polypeptide sequence encoded by the gene, differences in the amino acid sequence of the polypeptide, and the location in the polypeptide chain of the sequence differences. As a particular gene may have more than one sequence variance site, more than two allelic forms may exist in a population, for example, see Fig. 1 for exemplary target summaries showing multiple sequence variance sites.

Sequence variances can involve a difference in the sequence in which any of the four bases: adenine, guanine, thymidine (uracil in the context of RNA), or cytosine are substituted with another of the four bases or a change in the length of the sequence. Different classes of variances are recognized in the art. "Deletions" are variances in which one or more bases are missing from the sequence. "Insertions" are variances in which one or more bases are inserted into the sequence. It will be evident that the terms deletion and insertion refer to the variance in one sequence relative to another. "Transitions" are variances that involve substitution of one purine for the other or one pyrimidine for the other. "Transversions" are variances that involve substitution of a purine for a pyrimidine or a pyrimidine for a purine. Certain sequence variances can interfere with the normal function of the gene or its gene product and can be associated with disease; such variances are commonly referred to as mutations. Most

variances present in human populations are not associated with disease and are "normal" variants of the gene; such variances are commonly referred to as polymorphisms. In the present invention, specific variances are described from each of the classes described above in genes that are essential for cell survival or proliferation that can be the targets for allele-specific inhibitors for the treatment of cancer or noncancer proliferative disorders.

This invention provides inhibitors which are specific for at least one, but not all, allelic forms of a gene that encodes a gene product essential to cell growth or cell viability, for genes belonging to the specified categories of genes. The inhibitor may be active on the gene or gene product including the RNA transcript, protein product, or modifications thereof. Exposure to the inhibitor inhibits proliferation or kills cells which have undergone LOH of genes that are not inhibited by the drug and contain only an allelic form of the essential gene, its RNA transcript, or its protein product against which the inhibitor is targeted. Normal cells which contain two alternative alleles of the target genes, one of which is not inhibited by the specific inhibitor, are spared from the toxic effects of the inhibitor because the remaining activity of the allele which is not inhibited by the inhibitor is adequate to permit continued cell viability and growth. This differential effect of the inhibitor on cells with LOH of a targeted gene (*e.g.*, a cancer cell) and normal cells accounts for the high therapeutic index of the inhibitors of this invention for the treatment of cancer or non-cancerous, proliferative disorders characterized by LOH. Toxicity of the inhibitor to normal cells is therefore low, compared to most currently available anticancer and antiproliferative agents.

Thus, in accord with the strategy and target genes indicated above and described in the Detailed Description of the Preferred Embodiments, in a first aspect the invention provides methods for identifying inhibitors potentially useful for treatment of a proliferative disorder, *e.g.*, cancer. Such inhibitors are active on

specific allelic forms of target genes as identified herein. The method involves determining at least two allelic forms of such a gene encoding an essential gene product, and testing a potential allele specific inhibitor to determine whether the potential inhibitor is active on, *e.g.*, inhibits expression of, at least one of the allelic forms, but not all of those forms. If the potential inhibitor inhibits only a subset of the allelic forms of the particular essential gene, then it is an allele specific inhibitor. Preferably the difference in activity of the inhibitor for different allelic forms is between allelic forms which have a sequence variance at a particular site.

In many, or even most, cases an allele specific inhibitor discriminates between two allelic forms due to a particular single sequence variance between the allelic forms of the target gene. For example, ribozymes which target a single sequence variance site will preferentially cleave only one of the sequence variants for a particular single nucleotide variance. In this case, sequence variances at other sites will generally not affect the cleavage. In the Detailed Description of the Invention specific examples of proteins, small molecules, and oligonucleotides providing allele specific inhibition based on single sequence variances are described. Thus, in preferred embodiments an allele specific inhibitor discriminates between two allelic forms by discriminating a single sequence variance. As previously indicated, inhibitors can be targeted to either the nucleic acid or a polypeptide (where a nucleotide change results in an amino acid change). In particular embodiments, the allele specific inhibitor will recognize more than one linked sequence variances within a specific allele.

An "allele specific inhibitor" or "variance specific inhibitor" is a drug or inhibitor that inhibits the activity of one alternative allele of a gene to a greater degree than at least one other alternative allele. The difference in activity is commonly determined by the dose or level of a drug required to achieve a quantitative degree

of inhibition. A commonly used measure of activity is the IC50 or concentration of the drug required to achieve a 50% reduction in the measured activity of the target gene. Preferably an allele specific inhibitor will have at least twice the activity on the target allelic form than on a non-target allelic form, more preferably at least 5 times, still more preferably at least 10 times, and still more preferably at least 50 times, and most preferably at least 100 times. This can also be expressed as the sensitivities of the different allelic forms to the inhibitor. Thus, for example, it is equivalent to state that the target allelic form is most preferably at least 100 times as sensitive to the inhibitor as a non-target allelic form. The activity of an inhibitor can be measured either *in vitro* or *in vivo*, in assay systems that reconstitute the *in vivo* system, or in systems incorporating selected elements of the complete biological system. For use in inhibiting cells containing only the target allelic form rather than cells containing at least one non-targeted allelic form, the difference in activity is preferably sufficient to reduce the proliferation rate or survival rate of the cells having only the targeted allelic form to no more than one half of the proliferation rate or survival rate of cells having at least one non-targeted allelic form. More preferably, the fraction is no more than 1/5 or 1/10, and still more preferably no more than 1/20, 1/50, 1/100, or even lower.

In a related aspect, the invention provides inhibitors potentially useful for tumor, *e.g.*, cancer treatment, or treatment of other proliferative disorders. Such inhibitors are active on a specific allele of a gene which has at least two different alleles encoding an essential gene product in one of the target gene categories above. Such inhibitors can, for example, be identified by the above screening methods.

In a related aspect, the invention provides methods for producing inhibitors active on such specific allelic forms of belonging to one of the above categories genes by

identifying a gene encoding an essential gene product which has alternative allelic forms in a non-tumor cell and which undergoes LOH in a tumor cell, screening to identify an inhibitor which is active on at least one but less than all of the alleles of the gene, and synthesizing the inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a tumor in which tumor cells have only the allele on which the inhibitor is active.

In the context of this invention, the term "active on an allelic form" or "allele specific inhibitor" or "specific for an allelic form" indicates that the relevant inhibitor inhibits an allele having a particular sequence to a greater extent (preferably $\geq$ 2x) than an allele having a sequence which differs in a particular manner. Thus, for alleles for which a particular base position is identified, the inhibitor has a higher degree of inhibition when a certain base is in the specified position then when at least one different base is in that position. This means that for substitution at a particular base position, at least two of the possible allelic forms differ in sensitivity to an inhibitor. Usually, however, for a specific sequence variance site, the site will be occupied by one of only two bases. Further, if an inhibitor acts at the polypeptide level, and any of three bases may be present at a particular position in a coding sequence but only one of the substitutions results in an amino acid change, then the activity of the inhibitor would be expected to be the same for the two forms producing the same amino acid sequence but different for the form having the different amino acid sequence. Other types of examples can also occur.

The term "less active" indicates that the inhibitor will inhibit growth of or kill a cell containing only the allelic form of a gene on which the inhibitor is more active at concentrations at which it does not significantly inhibit the growth of or kill a cell containing only an allelic form on which the inhibitor is less active.

The term "drug" or "inhibitor" refers to a compound or molecule which, when brought into contact with a gene, its RNA transcript, or its gene product which the compound inhibits, reduces the rate of a cellular process, reduces the level of a cellular constituent, or reduces the level of activity of a cellular component or process. This description is meant to be illustrative of the understanding of the meaning of the term to those skilled in the art and not limiting. Thus, the term generally indicates that a compound has an inhibitory effect on a cell or process, as understood by those skilled in the art. Examples of inhibitory effects are a reduction in expression of a gene product, reduction in the rate of catalytic activity of an enzyme, and reduction in the rate of formation or the amount of an essential cellular component. The blocking or reduction need not be complete, in most cases, for the inhibitor to have useful activity. Thus, in the present invention, "inhibitors" are targeted to genes, their RNA transcript, or their protein product that are essential for cell viability or proliferation. Such inhibitors would have the effect of inhibiting essential functions, leading to loss of cell viability or inhibition of cell proliferation. In preferred embodiments, such inhibitors cause cell death or stop cell proliferation. In preferred embodiments of this invention, inhibitors specifically include a molecule or compound capable of inhibiting one or more, but not all, alleles of genes, their RNA transcript, or their protein product that are essential for cell survival or proliferation. The terms "inhibitor of a gene" or "inhibitor of an allele" as used herein include inhibitors acting on the level of the gene, its gene product, its RNA transcript, its protein product, or modifications thereof and is explicitly not limited to those inhibitors or drugs that work on the gene sequence itself.

Several types of inhibitors are generally recognized in the art. A "competitive" inhibitor is one that binds to the same site on the gene, its RNA transcript or gene product as a natural substrate or cofactor that is required for the action of the gene or gene product, and competitively prevents the binding of that substrate. An

"allosteric" inhibitor is one that binds to a gene or gene product and alters the activity of the gene or gene product without preventing binding of a substrate or cofactor. Inhibition can also involve reducing the amount of the gene, RNA transcript, or its protein product, and thus the total amount of activity from the gene in the cell. Such inhibition can occur by action at any of a large number of different process points, including for example by inhibiting transcription or translation, or by inducing the elimination of the gene, its RNA transcript, or its protein product where elimination may involve either degradation of the target or egress or export from the compartment in which it is active and the process of excretion or export. Inhibition can also be achieved by modifying the structure of the target, interfering with secondary modifications, or interfering with cofactors or other ancillary components which are required for its activity. Inhibitors can be comprised of small molecules or polymeric organic compounds including oligopeptides or oligonucleotides.

The term "active on a gene" or "targeted to a gene" indicates that an inhibitor exerts its inhibitory effect in a manner which is preferentially linked with the characteristic properties of a gene, its RNA transcript or its gene product. Such properties include, for example, the nucleotide sequence of the gene or transcribed RNA, the amino acid sequence or post-translational modifications of the protein product, the structural conformation of a protein, or the configuration of a protein or RNA with other cellular constituents (RNA, protein, cofactors, substrates, etc.) required for activity. Thus, in general these terms indicate that the inhibitor acts on the gene, its RNA transcript, its protein product, its gene product, or modifications thereof, or on a reaction or reaction pathway necessarily involving such a gene product to a greater extent than on genes or gene products generally.

A "reduction of the level of activity" of a gene product or allele product refers to a decrease in the functional activity provided by that product. This can be due to

any of a variety of direct causes, including for example, a reduction in the amount of a biologically active molecule present, a change in the structure or modifications of normally active molecules to produce inactive or less active molecules, blockage of a reaction in which the product participates, and blockage of a reaction pathway in which the product necessarily participates.

In another related aspect the invention provides methods for treating a patient suffering from a proliferative disorder in which an essential gene from one of the above categories has undergone loss of heterozygosity. The method involves administering a therapeutic amount of an allele specific inhibitor of such an essential gene to a patient whose normal somatic cells are heterozygous for that gene but whose tumor cells contain only a single allelic form of the gene. The inhibitor is active on the specific allele of the gene present in the tumor cells.

A "therapeutic effect" results, to some extent, in a measurable response in the treated disease or condition. Thus, a therapeutic effect can include a cure, or a lessening of the growth rate or size of a lesion such as a tumor, or an increase in the survival time of treated patients compared to controls, among other possible effects.

The term "therapeutic amount" means an amount which, when administered to a mammal, *e.g.*, a human, suffering from a disease or condition, produces a therapeutic effect.

In preferred embodiments of this treatment method, the method also involves determining whether the normal cells of the patient are heterozygous for the particular essential gene and determining whether tumor cells of the patient contain only a single allelic form of that gene. The determining may be performed on a variety of normal cells, such as blood or normal tissue, and on tumor cells.

Either or both of the normal cells and tumor cells may be cultured prior to the determination. The determination may also be carried out using cells retrieved from a frozen or preserved tissue specimen, *e.g.*, from pathological specimens of a patient's tumor and/or normal tissue preserved in a pathology laboratory. Also, the determining may be performed using a variety of techniques, which may, for example include one of more of: hybridization with an allele specific oligonucleotide probe, hybridization to a gridded set of oligonucleotides, restriction fragment length polymorphism, denaturing gradient gel electrophoresis, heteroduplex analysis, single strand conformation polymorphism, ligase chain reaction, nucleotide sequencing, primer extension, dye quenching, sequence specific enzymatic or chemical cleavage, mass spectroscopy, and other methods known in the art.

In a related aspect, the invention provides a method for preventing the development of cancer. The method involves administering to a patient having a precancerous condition or an early stage cancer or cancers an allele specific inhibitor targeted to an allele of an essential gene for which the normal somatic cells of the patient are heterozygous and which has undergone LOH in cells involved in the precancerous condition. In a case where the cells of the precancerous condition are not clonal from a single cell, the method involves subsequently administering to the patient a second allele specific inhibitor in an amount sufficient to inhibit and preferably kill cells with LOH in which an allele not targeted by the first inhibitor is the only remaining allele of the gene. In most cases, the second allele specific inhibitor will target the alternative allele of the gene targeted by the first inhibitor. However, the second inhibitor can also target an allele of a second essential gene which has undergone LOH. The second gene may have undergone LOH in the same deletion that affected the first gene due to their proximity on a chromosome, though this is not essential. Additionally, in other cases, allele specific inhibition of one of the alleles of each of 3, 4, or even

more target genes can be utilized in a serial manner (where the patient is heterozygous for each targeted gene). In this case the different target genes need not be tightly linked so that LOH of the various genes does not necessarily occur together. By using the serial inhibition of an allele of each of the target genes, it is possible to inhibit and preferably kill the full population of precancerous cells in which LOH has occurred. Thus, the net effect is essentially the same as if allele specific inhibitors of each of the two alternative alleles of one essential gene had been used.

In the context of the administration of multiple allele specific inhibitors, the terms "serial" or "subsequently" indicates that the administration of two or more inhibitors is sufficiently temporally separated so that normal somatic cells remain functional and are therefore able to survive and/or proliferate. Those skilled in the art will recognize that the required time will depend on various factors, such as clearance rate, type and extent of the effect of an inhibitor on normal cells, and additive cellular toxicity, and that appropriate timing can be routinely determined for particular selections of compounds.

In another related aspect, the invention provides a method for identifying a potential patient for treatment with an inhibitor active on a specific allele of an essential gene from one of the above categories. The method involves identifying a patient having a proliferative disorder characterized by LOH, *e.g.*, a cancer, whose normal somatic cells are heterozygous for the essential gene and determining whether tumor cells in the patient contain only a single allele of the gene. Thus, if the patient is normally heterozygous and the neoplastic cells contain only a single allele of the gene, then the patient is a potential patient for treatment with the inhibitor.

With respect to identifying patients with precancerous or oligoclonal proliferative

diseases characterized by LOH, and selecting appropriate allele or variance-specific inhibitors for such patients, in some cases it may not be practical to obtain samples of all proliferative lesions for LOH assays.. For example, atherosclerotic plaques in the aorta cannot routinely be sampled by biopsy, and dysplastic lesions in the cervix, colon, or bronchus can be multifocal. Therefore, allele specific inhibitors can be selected for such conditions based on previously established patterns of LOH for the condition, and on specific testing for heterozygosity in a given patient. Characteristic patterns of LOH involving specific chromosomes or chromosomal regions have been reported in the art (by Vogelstein's group and others) for premalignant changes in the colon, such as adenomatous polyps, polyps with dysplasia and polyps with carcinoma *in situ* (pre-invasive cancer) (Fearon, E. and B. Vogelstein). These studies demonstrate LOH on chromosomes 5q, 17p, and 18q in the earliest lesions. Similar studies have been performed for other premalignant conditions. It will be evident to one skilled in the art that similar studies can be readily performed on other conditions characterized by LOH using retrospective analysis of tissue from pathological specimens. The optimal regions for allele or variance specific targeting will be those which are affected by LOH in a high fraction of lesions and in a high fraction of patients. Preferably, at least 40% of lesions will have LOH for a specific target gene, more preferably 60, 80, or 90%, and most preferably 100%. However, it is not necessary that 100% of lesions show LOH for a successful treatment by allele specific inhibitors because 2,3,4, or even more inhibitors can be used in a combined approach to target an ever higher fraction of lesions, and because substantial therapeutic benefit may be achieved by inhibiting the proliferation of less than 100% of lesions.

In a related aspect, the invention provides a method for treating a patient having a proliferative disorder, *e.g.*, suffering from a cancer. The patient's normal somatic cells are heterozygous for an essential gene from one of the above categories, but the patient's cancer cells, or other abnormally proliferating cells,

have only a single allelic form of the gene. This method combines the identification and treatment methods described in the preceding aspects.

In another aspect, the invention provides a method for identifying a potential patient undergoing transplantation for treatment with an inhibitor active on a specific allele of an essential gene from one of the above categories. The method involves identifying a patient undergoing an allogenic transplantation in which the tissue of the donor contains at least one form of an essential gene that is different from those of the recipient. In a preferred aspect of this invention the donor or recipient is homozygous for an alternative form of an essential gene that differs from those present in the other. The term "homozygous" means that the two alleles of a gene present in somatic cells contain the same allele or alleles with identical sequence at at least one variant position that determines the activity of an allele specific drug. Such identification then allows methods of treating such patients by targeting the differing variances or allelic forms.

The term "allogenic" transplantation refers to transplantation of a tissue or cell fro the same species which contains different surface antigens than the recipient. In contrast, an "autologous" transplantation is one in which the patient receives their own tissues (commonly bone marrow) that contain identical surface antigens. The surface antigens are commonly those referred to as "histocompatibility" antigens or "HLA" antigens which allow the immune system to recognize the patient's own tissues from foreign tissue. In an allogenic transplant, the antigens on the donor tissue are different from those of the recipient. This can lead to an immune response in which the antigens on the transplanted tissue stimulate the patient's immune system to destroy or reject the transplanted tissue. Alternatively, in bone marrow transplantation, the antigens on the patient's normal tissue can stimulate the immune system constituted from the donor tissue to destroy the patient's normal tissues. This is termed "graft versus host disease" (GVH).

In a related aspect, the invention provides a method for treating graft versus host disease in allogenic transplantation in which an allele specific inhibitor is used to inhibit proliferation of donor cells, *e.g.*, to inhibit stimulation of the donor immune system. In preferred embodiments, the allele specific inhibitor is selected by identifying alternative variances or allelic forms of an essential gene that are present in the donor tissues but not the recipient. Therapy with a variance or allele specific inhibitor or inhibitors that recognizes both alleles of the essential gene that are present in the donor, but not both alleles of the same gene that are present in the recipient, can be used to suppress the immune response against the patient's tissues (GVH) without toxicity to these tissues. Most commonly, the donor tissue would be homozygous for a variance in the essential gene and the recipient would be homozygous to an alternative nucleotide or amino acid at a specificity determining site of variance. However, alternative combinations can also be used which result in at least one allelic form being present in the recipient which is not present in the donor cells, for example the donor could be homozygous and the recipient could be heterozygous for different allelic forms. As in other aspects described, a plurality of target genes can also be utilized.

In another aspect, the invention provides a method for enhancing engraftment of an allogenic bone marrow transplant in which an allele specific inhibitor is used to kill or suppress the patient's own bone marrow, providing "space" for engraftment of the donor cells within the marrow cavity. In preferred embodiments, the allele specific inhibitor is selected by identifying alternative forms of an essential gene that are present in the recipient but not the donor marrow. Therapy with an allele specific (generally a variance specific) inhibitor that recognizes both forms of the essential gene that are present in the recipient, but not both forms of the same gene that are present in the recipient, can be used to suppress the patient's own marrow without toxicity to the transplanted cells. It will be recognized by those in the art that this method can be used to reduce the

frequency of chimerism and increase the rate of success in engrafting an allogenic marrow.

"Chimerism" refers to a transplantation that is incomplete, leading to the proliferation of bone marrow progenitor cells derived from both the donor and recipient. Chimerism is generally an undesirable outcome that commonly results in gradual elimination of the graft due to competition with the patient's own cells. Allele specific inhibitors can be used to treat or prevent chimerism by selectively killing or suppressing proliferation of the patient's own cells without toxicity to the donor cells.

In another aspect, the invention provides a method for treating cancer in a patient receiving allogenic or autologous transplantation in which an allele specific inhibitor is used to kill or inhibit the growth of cancer cells without toxicity to the transplanted marrow. In one embodiment, in an autologous transplantation the allele specific inhibitor is selected to recognize one alternative allele of an essential gene remaining in the cancer cell due to LOH in patients who are heterozygous with two different alternative forms of the essential gene in their normal cells and in the autologous bone marrow graft. Treatment with such a drug will enable continuing therapy of cancer without suppression of the transplanted marrow. In an alternative embodiment, in an allogenic transplantation, therapy with an allele specific inhibitor that recognizes the one form of the essential gene that is present in cancer cells due to LOH in the recipient, but not an alternative form or forms of the same gene that are present in the recipient's normal cells and in the donor cells can be used to treat the cancer in the patient without toxicity to the transplanted cells. It will be recognized by those in the art that such therapy will enable more effective cancer therapy during and after transplantation. Moreover, such therapy would preserve the function of the immune system which is an important element in effective cancer therapy.

In a related aspect, the invention can be used *ex vivo* during autologous transplantation to eliminate malignant cells from the transplanted marrow. The principle of autologous bone marrow transplantation is that bone marrow can be harvested from a patient prior to high dose radiation or chemotherapy that would normally be lethal to the bone marrow. Following such therapy, the patient can then be treated by reimplantation of their own marrow cells to reconstitute the bone marrow and hematopoietic functions. An important limitation of this procedure is that bone marrow harvested prior to such therapy often contains many malignant cells, and that implantation of the harvested bone marrow often results in reseeding of the patient's malignancy. Various techniques for "purging" the bone marrow of such malignant cells have been described. These methods are focused on selecting "normal" bone marrow stem cells or progenitor cells that are within the harvested tissue for selective reimplantation. The present invention provides for an improved method for purging bone marrow of malignant cells using allele specific inhibitors of essential genes. The method involves identifying an essential gene with only one variant form remaining in the cancer cells due to LOH in patients who are heterozygous with two different alternative forms of the essential gene in their normal cells (and in the autologous bone marrow). The patient's bone marrow is then cultivated *ex vivo* using methods known in the art in the presence of an allele specific inhibitor that inhibits the allele that is present in the cancer cells, but not the alternative allele that is present in the heterozygous normal bone marrow. This treatment will result in killing of cancer cells within the graft, enabling selective reimplantation of normal cells. It will be recognized that one or more drugs could be used simultaneously or sequentially in this manner to achieve more efficient purging of cancer cells.

In another aspect, the present invention provides a method for sorting cells, for example for separating cancer cells from normal cells during an autologous bone marrow transplantation. The method utilizes a compound, preferably an antibody or

antibody fragment, which specifically binds to at least one but less than all the products of alleles which occur in a population of a particular gene which encodes a cell surface protein. Such a binding compound is used to bind with cells which express a targeted allele. If cancer cells from a patient who is heterozygous for that gene (having both a targeted allele and a non-targeted allele) have undergone LOH of the particular gene such that only the non-targeted allele is present in the cancer cells, then the binding compound can be used to bind to normal cells and to pull them out from a mixture of normal and cancer cells. This separation is possible because the binding compound will bind to the protein from the targeted allele of the gene expressed in the normal cells, but will not recognize and will not bind to the cancer cells as there is no product of the targeted allele present on those cells. Use of this method thus allows the isolation of normal cells, which can then be reintroduced to the marrow in an autologous transplant following anticancer treatment of the patient, thereby avoiding the problem of reintroduction of cancer cells. In this method, the targeted gene need not be an essential gene, or have any particular function. All that is needed is that the gene product be accessible or can be made accessible to the allele specific binding compound and that there be alternative allelic forms of the gene present such that the products can be distinguished by allele specific binding compounds and that the gene have undergone LOH between the normal cells and the cancer cells. However, it is also recognized that this method can also be used to separate any sets of cells which express different allelic forms of a gene where the gene products are accessible to allele specific binding compounds.

In preferred embodiments, the binding compound is immobilized, such as on a solid support, or can be caused to leave solution, such as by precipitation or by sandwich binding of the binding compound with a second binding compound, so that the bound cells are directly removed from the mixture. In other embodiments, the binding compound allows the recognition of the targeted cell, such that the cells can

be separated mechanically, for example using fluorescence activated cell sorting (FACS), or other cell sorting method as known to those skilled in the art. Also in preferred embodiments, the binding compound is an antibody or antibody fragment which retains allele specific binding. Such antibodies can be readily obtained by conventional methods as polyclonal or monoclonal antibodies after isolation of an appropriate antigen.

In another aspect, the invention provides a method for inhibiting growth of or killing a cell containing only one allelic form of a gene by contacting the cell with an inhibitor active on that allelic form. The gene has at least two sequence variants in a population, and belongs to one of the categories of essential genes described below. The inhibitor is less active on at least one other allelic form of the gene.

In preferred embodiments of the above aspects in which an allele specific inhibitor is used to inhibit a cell or to treat a patient, a plurality of different inhibitors may be used. Preferably different inhibitors target a plurality of different variances in a single target gene, or target variances in different target genes, or both. In particular embodiments a plurality of inhibitors is used simultaneously, in others there is serial administration using different inhibitors or different sets of inhibitors in separate administrations, which may be performed as a single set of administrations in which each set of inhibitors is administered once, or in multiple serial administrations in which each set of inhibitors is administered more than once. Such use of multiple inhibitors provides enhanced inhibition, which preferably includes killing, of the targeted cells. In addition, allele specific inhibitors as described can be used in conjunction with other treatments for diseases and conditions, including in conjunction with other chemotherapeutic agents such as other antineoplastic agents.

In a related aspect, an allele specific inhibitor can be used in conjunction with a conventional antiproliferative or chemotherapeutic agent or therapy, such therapies including radiation, immunotherapy, or surgery. In preferred embodiments the conventional therapy causes one or more genes within the cancer cell, or noncancer proliferative lesion, to be essential for cell survival that are would not be essential in the absence of said conventional therapy. For example, the treatment of cancer with radiation or alkylating agents makes efficient DNA repair essential for cell survival. In another example, depleting cancer cells of certain nutrients may make certain synthetic metabolic pathways essential. These examples are meant to be illustrative of the use of the present invention to those skilled in the art and not limiting. Further discussion and examples of the use of conditionally essential genes and their utilization in the methods of this invention are provided in the Detailed Description and the Examples.

In accord with the above aspects, in a further aspect the invention provides a pharmaceutical composition which includes at least one allele specific inhibitor. In preferred embodiments the composition includes at least one allele specific inhibitor and a pharmaceutically acceptable carrier. Such carriers are known in the art and some commonly used carriers are described in the Detailed Description below. Also in preferred embodiments the composition includes two, three, or more allele specific inhibitors, and may also include a pharmaceutically acceptable carrier. In other preferred embodiments, the composition includes at least one allele specific inhibitor and another antineoplastic agent, which need not be an allele specific inhibitor. The embodiments of this aspect may also optionally include diluents and /or other components as are commonly used in pharmaceutical compositions or formulations. In embodiments having a plurality of allele specific inhibitors, the inhibitors may target a plurality of different variances of a single target essential gene, or may target sequence variances of a plurality of different essential genes or combinations thereof.

In accord with the use of pharmaceutical compositions, the present invention also provides a packaged pharmaceutical composition comprising an allele specific inhibitor as described above, bearing a Food and Drug Administration use indication for administration to a patient suffering from a cancer or suffering from another proliferative disorder.

Determinations of essential gene heterozygosity and tumor cell LOH may be performed by a variety of methods, such as direct sequencing of known sequence variance sites and probe hybridization with variance specific probes. Thus, the invention also provides a nucleic acid probe at least 9, 12, 15 or 20 nucleotides in length, but preferably not more than 30 nucleotides, which will hybridize to a portion of a first allelic form of an essential gene in one of the above categories under specified hybridization conditions and not to a second allelic form under those hybridization conditions, the first and second allelic forms have a sequence variance within the complementary sequence. Preferably the probe is at least 12 nucleotides in length and is perfectly complementary to a portion of the first allelic form which includes a sequence variance site. The probe hybridizes under stringent hybridization conditions to the portion of the first allelic form and not to the corresponding portion of the second allelic form. This means that the probe does not bind to the second allelic form to an extent which prevents identification of the preferential specific binding to the first allelic form. The thermodynamics of the probe hybridization can be predicted to maximize the desired differential hybridization, providing optimization for probe length, sequence, structural modifications, and modifications to hybridization conditions.

The invention also provides nucleic acid probes or primers adjacent to the site of a variance that can be used to amplify a sequence containing the variant position to determine which variance is present at that position. Such probes or primers can readily be designed based on the sequences provided in the corresponding database

sequence entry or otherwise determined. The method of determining the variance can involve allele specific hybridization, sequencing or analysis of the amplified fragment by mass spectroscopy, SSCP, gene sequence database analysis, capillary electrophoresis, bindase/resolvase systems, or other methods known in the art. In a preferred embodiment, the amplified sequence spans more than one variant position and the method used for determining the variances identifies which variances are present at each position and combinations of variances that are present on each allele.

In preferred embodiments of the above aspects, the specific target allelic form has the characteristics as described above. Thus, for aspects in which the category of gene is specified, in preferred embodiments the gene belongs to a particular sub-category, for example, subcategories as specified in Table 1. Also in preferred embodiments, the gene is an identified target gene as listed in Table 1 or otherwise specified herein, including targeting utilizing the specified variances for exemplary genes described herein, singly or in combination in an allelic form. Also in preferred embodiments, the target gene is an allelic form having characteristics as specified above, for example is a gene which has a high frequency of heterozygosity and/or occurs in a chromosomal region which undergoes LOH in a cancer at a frequency as specified above. For aspects in which the target gene has a specified LOH frequency, the LOH frequency may be provided by published literature, inferred from the LOH of nearby genetic members, or independently determined, such as by the methods known in the art.

The use of conditionally essential genes for a number of applications is similar to the aspects above, but generally also involve an alteration of environment to make the gene essential and also provides additional aspects. For a conditionally essential gene, the essentiality may, but need not be absolute. Instead, in this context, the term "essential" means that the gene confers a significant advantage,

such that the growth or survival of the non-targeted cells is preferably at least 2x, more preferably 3x, 4x, 5x, 10x, or more as compared to the targeted cells.

Thus, similar to the above, the invention provides a method for identifying an inhibitor potentially useful for treatment of cancer or other proliferative disorder. The inhibitor is active on a conditionally essential gene, and the gene is subject to loss of heterozygosity in a cancer. The method includes identifying at least two alleles of a said gene which differ at at least one sequence variance site and testing a potential allele specific inhibitor to determine whether the potential inhibitor is active on at least one but less than all of the identified alleles. If the potential inhibitor inhibits expression of at least one but less than all of the alleles or reduces the level of activity of a product of at least one but less than all of the alleles, this indicates that the potential allele specific inhibitor is, in fact such an allele-specific inhibitor inhibitor.

In preferred embodiments of this and the various aspects described below, the conditionally essential gene is one of the exemplary genes presented in the table of conditionally essential genes or in the examples.

Similar to other types of target genes described above, the invention provides inhibitors, methods for producing inhibitors, pharmaceutical compositions, methods for identifying potential patients, probes, and primers which target or recognize alleles of a conditionally essential gene or utilize inhibitors which target such genes.

The invention also provides methods for preventing the development of cancer, methods for treating a patient suffering from a cancer, and methods for inhibiting growth of a cells as described above except that the targeted cells are subjected to an altered condition such that the gene becomes essential.

In still another aspect, not requiring the use of allele specific inhibitors, but still utilizing information about sequence variance or allelic differences between normal somatic cells and cancer cells in a patient, the invention provides a method for selecting a patient for treatment with an antiproliferative treatment. The method includes the following steps: determining whether normal somatic cells in a potential patient are heterozygous for an essential or conditionally essential gene, where a first allelic form of the gene is more active than a second allelic form, and where a reduction in the activity of the gene in a cell increases the sensitivity of that cell to an antiproliferative treatment; and determining whether cancer cells from the patient have only the second allelic form of the gene. If the somatic cells are heterozygous and the cancer cells have only the second allelic form, this indicates that the patient is suitable for treatment with the antiproliferative treatment because the cancer cells will be more sensitive to the antiproliferative treatment. In preferred embodiments, the antiproliferative treatment is radiation or administration of a cytotoxic drug.

In a related aspect, the differences between the normal somatic cells and the cancer cells in a patient are used in a method for selecting an antiproliferative treatment for a patient suffering from a cancer. This method involves determining whether there will be a differential effect of the prospective treatment on the cancer cells as compared to the normal cells based on a differential response of the cancer cells due the presence in the cancer cells of only the less active form of a conditionally essential gene which is present in two alternative allelic forms with differing activities in the somatic cells. The method thus involves determining whether normal somatic cells in a potential patient are heterozygous for an essential or conditionally essential gene which reduces the sensitivity of cells to an antiproliferative treatment. As noted, a first allelic form of the gene is more active than a second allelic form, and a reduction in the activity of the gene in a cell increases the sensitivity of that cell to the prospective antiproliferative treatment;

and determining whether cancer cells of said patient have only the second, less active, allelic form of the gene. If these factors are present, this indicates that the proposed treatment is suitable for that patient.

In preferred embodiments of above aspects, a conventional therapy acts on a protein or other molecular target in the same pathway as the allele specific inhibitor. As an example, the antineoplastic drug hydroxyurea, which inhibits ribonucleotide reductase (RR), can be used in conjunction with an allele specific inhibitor of RR subunit M1 or M2 or another gene that encodes a product important in nucleotide synthesis. Similarly, the antiproliferative drug methotrexate inhibits the enzyme dihydrofolate reductase (DHFR), and can be used with allele specific inhibitors of DHFR that would result in a differential methotrexate effect on cancer tissues compared to normal proliferating tissues. Alternatively, methotrexate can be used with allele specific inhibitors of other genes important in folate metabolism to achieve an enhanced cancer cell specificity for methotrexate. Similarly, the anticancer drug 5-fluorouracil and related compounds can be administered together with an allele specific inhibitor of thymidylate synthase (TS) in a patient heterozygous for TS and with LOH at the TS gene in proliferating cells, e.g., cancer cells. Alternatively, an allele specific inhibitor of 5-FU degradation or metabolism can be administered with 5-FU. For example, the enzyme dihydropyrimidine dehydrogenase, which catalyzes the first and rate limiting step in 5-FU catabolism would have the effect of potentiating 5-FU action in cancer cells due to their lesser ability to metabolically inactivate 5-FU. One skilled in the art will readily recognize that similar methods can be used with other conditionally essential genes, including specific genes listed in the table of conditionally essential genes.

Some conditionally essential genes occur in active and less active, or nearly inactive allelic forms. Further, some cancer patients are heterozygous for active and less active forms in their normal tissues, but due to LOH, their cancer cells contain only

the less active allelic form. As describe above, such patients can be identified by a diagnostic test of their normal cells and cancer cells. Such a test will identify which patients should be treated with a specific treatment, such as a particular drug or radiation treatment or other treatment. Such a therapy, which is not allele specific, would nonetheless have cancer specific effects due to the LOH-determined difference in the ability of the cancer cells to respond to the cytotoxic or cytostatic effects of therapy.

For example, patients with Ataxia Telangiectasia are homozygous for mutant alleles of the ATM gene. Such individuals are hypersensitive to radiation therapy or radiomimetic drugs. Heterozygotes for normal and mutant ATM are normal and have been estimated to account for 0.5-1% of the North American population, but, due to an increased risk of caner, may account for up to 5% of some cancers, for example, breast cancer. The ATM gene maps to chromosome 11q23, a region frequently affected by LOH in breast and other cancers. In breast cancers arising in ATM heterozygotes in which the more active (normal) ATM allele is lost in cancer tissue due to LOH, treatment with radiation or radiomimetic drugs would be differentially toxic to cancer cells. It has been shown that ATM heterozygotes are less sensitive to such treatments than ATM mutant (less active) homozygotes. Such use of an LOH diagnostic procedure to select appropriate antineoplastic therapy represents a change from the current procedures which are based solely on tissue origin, grade, and stage of cancer.

In such an approach, preferably the difference in activity between more active and less active allelic forms is at least 2x, more preferably at least 3x, 4x, or 5x, and most preferably at least 6x, 10x, or even more.

Preferably a target conditionally essential gene is one such that at least 0.1%, 0.5%, 1% or 5%, or the higher rates as stated above, of a population is

heterozygous for a particular sequence variance

Additional specific genes within the categories or subcategories described which are potentially useful for allele specific therapy can be readily identified by those skilled in the art using the methods described herein and/or using information available to those familiar with cellular genetics and tumor biology. In particular such genes can be identified and/or obtained by identifying essential genes, determining whether the gene contains sequence variants in a population, determining whether the gene undergoes LOH in one or more tumors or other proliferative disorders. Genes having these characteristics can then be used for identifying allele specific inhibitors and evaluated for use in the other methods of this invention. Such procedures are routine, as is shown by the Detailed Description of the Preferred Embodiments below, including the Examples.

In preferred embodiments of the above methods and inhibitors involving particular target genes or classes or categories of genes, the inhibitor or potential inhibitor is a ribozyme which is designed to specifically cleave a particular target allelic form of a gene (*i.e.*, a nucleotide sequence such as mRNA).

The ribozyme is designed to cleave the nucleotide (*e.g.*, RNA) sequence at a position in the nucleotide chain of the target allelic form at or near the position of a sequence variance. Usually the ribozyme will have a binding sequence which is perfectly complementary to a target sequence surrounding the sequence variance site. Preferably, the ribozyme does not consist of only ribonucleotides, and therefore includes at least one nucleotide analog or modified linkage. In preferred embodiments the ribozyme has a hammerhead or hairpin motif, but may have other structural motifs as known to those skilled in the art..

The term "ribozyme" refers to a catalytic RNA molecule, including those

commonly referred to as hammerhead ribozymes and hairpin ribozymes, generally having an endonuclease activity, but includes catalytic RNA molecules, catalytic DNA molecules (DNAzymes), and derivatives of such molecules unless indicated to the contrary. In particular, as understood by those skilled in the art, ribozymes may incorporate a variety of nucleotide analogs, modified linkages, and other modifications.

In connection with ribozymes, "target sequence" refers to a nucleotide sequence which includes a binding site and a cleavage site for a ribozyme. For use in this invention, preferably a gene having a ribozyme target sequence exists in two allelic forms in normal somatic cells of a patient. The two allelic forms differ in nucleotide sequence within the target sequence, *i.e.*, have a sequence variance within the target sequence.

Also in connection with ribozymes, the term "specifically cleaves" means that a particular ribozyme will cleave a target sequence to a greater extent than it will cleave a different sequence. For allele specific ribozymes, this means that for two allelic forms having a sequence variance in the target sequence, preferably the ribozyme will cleave one of the allelic forms more efficiently than the other. Those skilled in the art will understand that the target discrimination can be provided by base differences within the ribozyme binding sequence of the substrate at or close to the cleavage site.

Similarly, in preferred embodiments the inhibitor or potential inhibitor is an oligonucleotide, e.g, an antisense oligonucleotide, preferably at least partially an oligodeoxyribonucleotide. The antisense oligonucleotide is complementary to a sequence which includes a sequence variance site. Usually, though not necessarily, the antisense oligonucleotide is perfectly complementary to a sequence of the target allelic form which includes a sequence variance site. The antisense

oligonucleotide preferably is at least twelve nucleotides, more preferably at least seventeen nucleotides in length. In some cases the antisense oligonucleotide may advantageously be longer, for example, at least 20, 25, or 30 nucleotides in length. Also in preferred embodiments, the oligonucleotide is no longer than 20, 25, 30, 35, 40, or 50 nucleotides The optimal length will depend on a number of factors, which may include the differences in binding free energy of the oligonucleotide to the target sequence as compared to binding to the non-target allelic form, *i.e.*, the non-target sequence variant, or the kinetics of nucleic acid hybridization. The oligonucleotide preferably contains at least one nucleic acid analog or modified linkage. Such complementary oligonucleotides may function in various ways, and those skilled in the art will know how to design the oligonucleotide accordingly. Such functional mechanisms include, but are not limited to direct blocking of transcription of a gene by binding to DNA (*e.g.*, high affinity antisense, including triple helix), direct blocking of translation by binding to mRNA, RNaseH mediated cleavage of RNA or other RNAase mediated cleavage, and binding-induced conformational changes which block transcription or translation or alter the half-life of mRNA. Triple-helix modes of action include the formation of a triple-helical structure between the two strands of genomic DNA and an antisense molecule, *i.e.*, anti-gene strategy, or between an RNA molecule and an antisense oligonucleotide which loops back to contribute two of the three strands of the triple helix, or between an RNA and an antisense where the RNA provides two of the three strands of the triple helix.

The term "oligonucleotide" refers to a chain molecule comprising a plurality of covalently linked nucleotides as recognized in the art. The oligonucleotide preferably has about 200 or fewer backbone units corresponding to nucleotide subunits, more preferably about 100 or fewer, still more preferably about 80 or fewer, and most preferably about 50 or fewer. An oligonucleotide may be modified to produce an oligonucleotide derivative. Unless indicted otherwise the

term "oligonucleotide" includes "oligonucleotide derivatives".

A large number of nucleic acid modifications are known in the art which may be used in the nucleic acid molecules of the present invention, thereby producing "nucleic acid derivatives" or "oligonucleotide derivatives". Such modifications can be used, for example, to enhance resistance to degradation by nucleases or to modify functional characteristics such as binding affinity. In preferred embodiments, the ribozyme, antisense oligonucleotide, or other nucleic acid molecule contains at least one modified linkage, including but not limited to phosphorothioate, phosphoramidate, methylphosphonate, morpholino-carbamate, and terminal 5'-5' or 3'-3' linkages. Also in preferred embodiments, the nucleic acid molecule contains at least one nucleotide analog. Such analogs include but are not limited to nucleotides modified at the 2' position of the ribose sugar, *e.g.*, 2'-O-alkyl (e.g., 2'-O-methyl or 2'-methyoxyethoxy) or allyl, 2'-halo, and 2'-amino substitutions, and/or on the base (e.g., C-5 propyne pyrimidines), and analogs which do not contain a purine or pyrimidine base, and includes the use of nucleotide analogs at the terminal positions of a nucleic acid molecule. Preferably a 2'-O-alkyl analog is 2'-O-methyl; preferably a 2'-halo analog is 2'-F.

A specific embodiment of this invention is the use of hybrid oligonucleotides that contain within a linear sequence two different types of oligonucleotide modifications. In a particular embodiment, these modifications are used such that a segment of the oligonucleotide that hybridizes to the sequence variance is RNAase sensitive, but other segments are not RNAase sensitive.

Other modifications may also be used as are known in the art, such as those described in connection with antisense and triple helix in: Crooke & Bennett, 1996, *Annual Rev. Pharm. and Toxicol.* 36:107-129; Milligan et al., 1993, *J. Med. Chem.* 36:1923-1937; Reynolds et al., 1994, *Proc. Nat. Acad. Sci. USA*

91:12433-12437; and McShan et al., 1992, *J. Biol. Chem.* 267-5712-5721, which are hereby incorporated by reference. An additional modification useful for delivery of oligonucleotides is complexation of oligonucleotides with nano-particles, as described in Schwab et al., 1994, *Proc, Nat. Acad. Sci. USA* 91:10460-10464. As described further below, oligonucleotides may be complexed with other components known in the art which provide protection and/or enhanced delivery for the oligonucleotides, and may be useful for either gene delivery or for delivery of non-coding oligonucleotides.

Thus, "derivatives of nucleic acid inhibitors" include modified nucleic acid molecules which may contain one or more of: one or more nucleotide analogs, including modifications in the sugar and/or the base, or modified linkages, base sequence modifications, and insertions or deletions, or combinations of the preceding. Other derivatives are also included as are known in the art.

Similarly, in preferred embodiments the inhibitor or potential inhibitor is an antibody, preferably a monoclonal antibody, which may be complexed or conjugated with one or more other components, or a fragment or derivative of such an antibody. It is recognized in the art that antibody fragments can be produced by cleavage or expression of nucleic acid sequences encoding shortened antibody molecule chains. Such fragments can be advantageously used due to their smaller size and/or by deletion of sites susceptible to cleavage. In addition, derivatives of antibodies can be produced by modification of the amino acid moieties by replacement or modification. Such modification can, for example, include addition or substitution or modification of a side chain or group. Many modifications and biological effects of such modifications are known to those skilled in the art, and may be used in derivatives of antibodies in accord with those biological effects. Such effects can include, for example, increased resistance to peptidases, modified transport characteristics, and ability to carry a ligand or other

functional moiety. In preferred embodiments, the antibody is a humanized antibody from a non-human animal, *e.g.*, a humanized mouse or rabbit antibody. Many instances of monoclonal antibodies that distinguish protein differing by a single amino acid are known in the art.

An inhibitor may also be an oligopeptide or oligopeptide derivative. Such peptides may be natural or synthetic amino acid sequences, and may have modifications as described for antibodies above. In general, an oligopeptide will be between about 3 and 50 residues in length, preferably between about 4 and 30, more preferably between about 5 and 20 residues in length.

In other embodiments, the inhibitor is a small molecule, for example, a molecule of one of the structural types used for conventional anticancer chemotherapy.

By "small molecule" or "low molecular weight compound" is meant a molecule having a molecular weight of equal to or less than about 5000 daltons, and more preferably equal to or less than about 2000 daltons, and still more preferably equal to or less than about 1000 daltons, and most preferably equal to or less that about 600 daltons. In other highly preferred embodiments, the small molecule is still smaller, for example less than about 500, 400, or 300 daltons. As well known in the art, such compounds may be found in compound libraries, combinatorial libraries, natural products libraries, and other similar sources, and may further be obtained by chemical modification of compounds found in those libraries, such as by a process of medicinal chemistry as understood by those skilled in the art, which can be used to produce compounds having desired pharmacological properties.

In connection with the gene sequences or subsequences of gene sequences or primer sequences as described herein, the sequences listed under the accession

number are believed to be correct. However, the genes can be readily identified and the invention practiced even if one or more of the specified sequences contain a small number of sequence errors. The correct sequence can be confirmed by any of a variety of methods. For example, the sequence information provided herein and/or published information can be used to design probes for identifying and isolating a corresponding mRNA. The mRNA can be reverse transcribed to provide cDNA, which can be amplified by PCR. The PCR products can then by used for sequencing by standard methods. Alternatively, cDNA or genomic DNA libraries can be screened with probes based on the disclosed or published gene sequences to identify corresponding clones. The inserts can then be sequenced as above. If complete sequence accuracy is desired, such accuracy can be provided by redundant sequencing of both DNA strands. Those skilled in the art will recognize that other strategies and variations can also be used to provide the sequence or subsequence for a particular gene.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows seventeen gene-specific Target Gene Summary Tables which show variances detected in some of the exemplary genes described as examples in the specification. Those genes are:

Sodium, potassium ATPase
CTP synthetase
Ribonucleotide reductase M1 subunit
Thymidylate synthase

Alanyl tRNA synthetase
Cysteinyl tRNA synthetase
Glutamyl-prolyl tRNA synthetase
Glutaminyl tRNA synthetase
Lysyl tRNA synthetase
Threonyl tRNA synthetase
Ribosomal protein S14
Eukaryotic initiation factor 5A
Replication protein A, 70 kD subunit
Replication protein A, 32 kD subunit
RNA Polymerase II, 220 kD subunit
TATA associated factor IIH
Dihydropyrimidine dehydrogenase

These tables show, in the title, the name of each gene, its chromosome location and the Varia ID number. The horizontal section of the table displays, from left to right, the name of the primers used to amplify the polymorphic segment, the number of the polymorphic nucleotide (the numbering corresponds to the GenBank accession number reported in the central box under 'Sequence from:') and the two alternative sequences at the variant site. Then, under columns 1 - 36, the genotypes of 36 lymphoblastoid cell lines are given, followed by the frequency of heterozygotes ('het rate'), a 'Comments' section which describes any unusual aspects of the variances, a 'Location' section which reports the location of any variances and the inferred effect on amino acid sequence, if any, and a 'Race specific heterozygosity' section which reports frequency of heterozygotes in any racial groups with particularly high heteroxygosity levels. Below the 'Genotypes of 36 unrelated individuals' section the racial or ethnic identity of the subjects is shown (see legend in box at right: 'Ethnic & racial groups surveyed'). The sequence surrounding the variances is shown in the box at bottom left, with the

location of the variant base marked in bold type.

Fig. 2 is a schematic showing the practical flow of the SSCP technique as used for exemplary target genes. This flow chart, in conjunction with the description of the SSCP technique in the Detailed Description, demonstrates how sequence variances of the exemplary genes were identified. In conjunction with published descriptions of the SSCP technique, one skilled in the art can thus readily use SSCP to identify sequence variances in other genes within the scope of this invention.

Fig. 3 is a table describing the extent and distribution of loss of heterozygosity throughout the genome for a number of cancers as reported in the literature. The table is divided into 41 sections, one for each fo the chromosomal arms for which there is information about LOH frequency. (There is no information for the short arm [called the p arm] of chromosomes 13, 21 or 22, all of which are very short and contain mostly repetitive DNA.) In each of the 41 sections there is a list of polymorphic loci (sites) that have been tested for LOH in one or more cancer types. The loci are ordered, to the extent that present information allows, from the telomeric end of the short arm of the chromosome to the centromere (p arm tables), or from the centromere to the telomeric end of the long arm of the chromosome (q arm tables). Many chromosomes have not yet been well studied for LOH, so the absence of data on LOH in a particular cancer type on a particular chromosome arm should not be construed as indicating no LOH. It may simply indicate no good LOH studies have yet been published. The Loss of Heterozygosity Table is explained in detail below.

Column 1 Chromosomes, when stained with dyes such as giemsa, have alternating dark and light staining bands. These bands are the basis of chromosome nomenclature. Many of the markers used for LOH studies have been assigned to

specific chromosome bands, or can be inferred as likely to belong to specific bands based on other information. The 'unknown' notation in this column indicates that the paper from which the data was obtained (column 7) did not provide chromosome band information. In such cases other information has generally been used to order the data, however the order of some markers remains uncertain.

Column 2 LOH studies are performed with specific DNA markers or probes (for Southern blotting) or with DNA primers (if polymerase chain reaction was used) from a specific site, or locus, on a chromosome. The name of the marker, locus or probe used to perform each LOH assay is given in the second column of the Table, under 'Marker'. In the Table the markers are listed in their likeliest order along the chromosome, from the telomere of the p arm to the centromere for the p arm tables, and from the centromere to the telomere of the q arm for the q arm tables.

Columns 3, 4 & 5 The total number of cancers evaluable for LOH at the specific marker shown in column 2 (in the paper cited in column 7) are shown in column 3, 'Total'. This is generally the number of patients that were heterozygous for the marker in their normal DNA. Column 4, 'Cases w/LOH', shows the number of patients with LOH at the DNA marker. Column 5, 'LOH Freq', is the quotient of column 4 divided by column 3, giving the fraction of patients with LOH at the indicated marker.

Column 6 The type of cancer studied is indicated under the heading 'Tumor Type'. In some cases more detailed clinical information on cancer subtype or clinical stage is available in the paper cited in column 7.

Column 7 The literature citation, or 'Reference', from which the data was drawn. The references are provided in a compact form consisting of journal abbreviation (see the list of journal abbreviations below), volume and page.

Note

Studies of allele loss in benign neoplasms or in non-neoplastic conditions are not included in this table.

Journal Abbreviations for Literature Cited in the Table

The abbreviations used in the Tables are as follows:

AJHG = American Journal of Human Genetics
AJP = American Journal of Pathology
B = Blood
BJC = British Journal of Cancer
C or CA= Cancer
CCG = Cancer Cytogenetics
CGC = Cell Genetics and Cytogenetics
CL = Cancer Letters
CR = Cancer Research
CSurv = Cancer Surveys
EJC = European Journal of Cancer
G or GE = Genomics
GCC = Genes, Chromosomes & Cancer
GO = Gynecological Oncology
HG = Human Genetics
HMG = Human Molecular Genetics
IJC = International Journal of Cancer
JAMA = Journal of the American Medical Association
JJCR = Japanese Journal of Cancer Research (Gann)
JNCI = Journal of the National Cancer Institute
JU = Journal of Urology

Lan = Lancet

LI = Laboratory Investigation

N = Nature

NEJM or NEJ = New England Journal of Medicine

O = Oncogene

PN or PNAS = Proceedings of the National Academy of Sciences

S = Science

This data base thus identifies sites and regions of LOH associated with the particular identified cancers, including high frequency LOH chromosomal arms as well as the identified smaller regions associated with the particular markers. Both as indicated in the Summary and Detailed Description, LOH information such as this identifies essential genes mapping to those LOH regions as likely potential target genes because of the high probability that an essential gene in such a region undergoes LOH at frequencies similar to the marker. Such gene identification thus further identifies particular cancers which can potentially be treated with inhibitors targeting sequence variances in those essential genes.

The database provided shows information which is contained in published references dealing with cancer LOH. Those skilled in the art will recognize however that similar information can be readily obtained from the published literature in relation to other cancers and other neoplastic disorders. Thus this table demonstrates that one skilled in the art can readily identify regions of high frequency LOH for other such disorders and cancers, and can further readily identify essential genes which are potential targets for variance specific inhibition and the treatment of the corresponding condition and in other aspects of this invention.

Fig. 4 is a table summarizing the results in Fig. 3 by chromosome arm. Data for

all loci on each chromosome arm has been summed in a single statistic for LOH frequency on that chromosome arm.

Fig. 5 is a Target Variances by Field Table, which summarizes information on DNA sequence variances in selected genes from the Target Gene Table (Table 1), and is organized into groups of related genes that parallel the fields in the Target Gene Table.

- The heading at the top of each category of essential genes shows a number and a subcategory name. The number indicates which of the six principal categories of essential genes the subcategory belongs to (e.g. genes required for cell proliferation is category 1, genes required to maintain inorganic ions at levels compatible with cell growth or survival is category 2, etc.).
- Below the heading is a sentence on 'Validation' which briefly refers to some of the data which shows that genes in the subcategory are essential. Summary information on target gene variances is then listed, with five columns of data.
- The first column gives the Variagenics gene ID number, which serves as a cross reference to the Target Variances Table (see below), where more detailed information on variances can be found.
- The second column lists gene names. (The GenBank accession number in column 5 may be a more reliable way to identify genes.)
- The third column lists the number of variances found. These variances were detected by a variety of experimental and informatics based procedures described in the examples. Many variances were detected by two independent methods (e.g. informatics based detection and T4 endonuclease VII detection). A molecular description of the variances is provided in the Target Variances Table (see below).
- The fourth column lists the chromosome location of the target gene, if known. Knowledge of the chromosome location permits assessment of the

cancers in which LOH would be expected to affect the target gene. (See the Loss of Heterozygosity Tables for a detailed listing of LOH by chromosome region.)

- The fifth column lists the GenBank accession number of the target gene. (Some of the genes specified in the Table do not yet have GenBank accession numbers. For example, genes encoding several human tRNA synthetases and ribosomal subunits have not yet been cloned, although their existence can be inferred from genetic and biochemical studies and from phylogeny.

Fig. 6 is identical to Fig. 5, except that it concerns exemplary conditionally essential genes rather than generally essential genes.

Fig. 7 is a Target Variances Table shows molecular details of exemplary variances identified by Variagenics in exemplary target genes. There are six columns in the Table.

- The first column gives the Variagenics gene ID number, which serves as a cross reference to the Target Variances by Field Table (see above), where information on gene location and GenBank accession number are provided. After the ID number is a decimal point and then a list of one or more integers (on successive lines), which are the (arbitrary) numbers of the specific variances identified. Between one and 13 variances were identified per target gene. Information on different target genes is separated by dashed horizontal lines.
- The second column lists the location of the variance - specifically the number of the nucleotide at which variation was observed. The nucleotide number refers to a cDNA sequence of the target gene which can be retrieved using the GenBank accession number provided in the Target Variances by Field Table.
- The third column lists the two variant sequences identified at the specified

nucleotide. The variant nucleotides are bracketed and in bold font separated by a slash. Ten nucleotides of flanking sequence are provided on either side of the variance to localize the variant site unambiguously. (In the event of a conflict between the nucleotide number specified in column 2 and the sequence specified in column 3 the latter would rule as the correct sequence.) These variances were detected by a variety of experimental and informatics based procedures described in the examples. Many variances were detected by two independent methods (e.g. informatics based detection and T4 endonuclease VII detection).

- The fourth and fifth columns (headed '# Varia 1' and '# Varia 2') provide the number of occurrences of variance 1 and 2, respectively, where variance 1 is the first and variance 2 the second of the bracketed nucleotides in column three. In both the fourth and fifth columns there are two numbers. The first number reports the number of occurrences of the variance. 'Occurrences' include ESTs identified during informatics based analysis, or variances identified experimentally by analysis of human cell lines, or both. The second number, inside parentheses, reports the number of individuals in whom the occurrences were detected. An 'individual' means either a cell line (analyzed experimentally) or a cDNA library created from one individual (but from which many ESTs for the target gene may have been sequenced). Thus if the first number is 15 and the second number is 11 then there were 15 occurrences of the variance (a combination of 15 ESTs and/or 15 experimentally identified alleles) in a total of 11 cDNA libraries and/or cell lines.
- The fifth column provides annotation on the variances, particularly concerning the location of the variant site in the cDNA and the effect of the DNA sequence variance on the predicted amino acid sequence, if any. 5' UT = 5' untranslated region; 3' UT = 3' untranslated region; silent = variance lies in coding region by does not affect predicted amino acid

sequence; ND = analysis not done; Thr -> Asn = specific amino acid substitutions, inferred from the nucleotide sequence variance, are provided. Similar information can be readily obtained for additional genes using the methods described or as known to those skilled in the art.

Figures 9-15 correlate with Example 31.

Fig. 9 is a bar graph showing the number of T24 human bladder cancer cells surviving 72 hours after transfection with antisense oligonucleotides. Anti-ras is an oligonucleotide known to have antiproliferative effects against T24 cells. This oligonucleotide exhibits inhibition comparable to the anti-RPA70 oligonucleotide. Anti-herpes and an oligonucleotide with a scrambled sequence are shown as controls. This experiment demonstrates that RPA70 is an essential protein.

Cells were plated in six well dishes 24 hr prior to the experiment and transfected at approximately 50-70% confluency with various phosphorothioate oligomers at 400 nM. An oligomer:lipofectin ratio of 3 ug Lipofectin/ml Optimem/100 nM Phosphorothioate oligomer was used for all transfections. Prior to transfection the cells were washed once with room temp Optimum (BRL) and then Lipofectin diluted into Optimem was added to the cells. After addition of the lipofectin the antisense oligomers were immediately added. After a five hour incubation the medium was removed from the cells and replete medium added. The cells were allowed to recover, trypsinized, and cell number was determined at 72 hr by counting with a hemocytometer. Each bar represents two different determinations of cell number for each of three triplicate samples.

Fig. 10 is a Northern Blot demonstrating specific suppression of RPA70 mRNA levels in two cell lines with opposite genotypes. RPA70 in Mia Paca II cells matches the 13085 oligomer while RPA70 in T24 cells matches the 12781

oligomer. The 13706 oligomer is a random sequence control. Cells were plated in P100 dishes transfected as described in figure legend 11. Twenty-four hours after the addition of the indicated oligomers, RNA was recovered from the cells by the SDS-Lysis method (Peppel, K and Baglioni, C. *Biotechniques,* Vol. 9, No. 6, pp 711-7131, 1990). For Northern Blots 5-10 ug RNA per well was loaded onto a formaldehyde gel, electrophoresed and transferred to BioRad Zeta Probe GT. After baking (30 min at 80 C in a vac oven) the blot was probed for specific mRNA using a random primed 32P-labeled cDNA specific for RPA 70.

Fig. 11 is a Northern blot showing allele-specific Suppression of RPA 70 mRNA in T24 and Mia Paca II cells. Cells were plated in P100 dishes, transfected, and RPA 70 mRNA levels measured as previously described. T24 cells contain the genotype targeted by oligomer 12781. Mia Paca II cells are homozygous for the variance targeted by oligomer 13085. 12781 is a 20 nucleotide long phosphorothioate oligomer which targets RPA70 in T24 cells. 13085 is an 18 nucleotide long phosphorothioate oligomer which targets RPA70 in Mia Paca II cells. The lower half of the figure shows the EtBr stained gel of total RNA probed by Northern Blot.

Fig. 12 is two graphs showing that the proliferation of two cell lines homozygous for different variant forms of the RPA70 gene is inhibited to a greater degree by matched oligonucleotides than by oligomers having a single base mismatch. Cell proliferation was measured by BrdU incorporation in cellular DNA. Transfections were performed on consecutive days and BrdU incorporation measured 24 hours after the last transfection (see figure legend 9). Oligomer 12781 targets the variance contained in A549 cells and is mismatched relative to the genotype of Mia Paca II cells. Oligomer 13085 targets the variance contained in Mia Paca II cells and is mismatched relative to the genotype of A549 cells.

Fig. 13 is a graph showing Inhibition of BrdU incorporation in A549 cells by antisense oligonucleotides against the RPA 70 gene. Cells were transfected, as described previously, with a matched oligonucleotide (12781) or an oligonucleotide with one mismatch (13085). The oligonucleotide concentration was 400 nM with specific oligomer diluted with a random oligonucleotide. Cell proliferation was measured by BrdU incorporation after two transfections. Twenty-four hours after the first transfection the cells were transfected identically. Twelve hours after the second transfection BrdU was added to the cells and BrdU incorporation was assayed after a 12 hour incubation. BrdU incorporation was measured by ELISA (Boehringer Mannheim) with the following changes: Volumes were increased to assay BrdU incorporation in 6 well dishes. 1000 μl of fix, 750 ul of antibody, and 1000 ul of substrate. A portion of the samples were transferred to a 96 well dish (in triplicate) and read at 405 nm on a plate reader.

Fig. 14 is a graph showing antiproliferative/cytopathic effects of antisense oligonucleotides against the RPA70 gene in A549 cells. Cells were transfected on three consecutive days with a matched oligonucleotide (12781) or an oligonucleotide containing a one base mismatch (13085). Following the last transfection the cells were allowed to recover three days. Cell number was quantified by Sulforhodamine B staining (Molecular Probes). Volumes were increased to accommodate the assay in 6 well dishes. Fixation 1.25 ml, stain 750 ul, solubilizer 1 ml. A portion of the samples were then transferred to a 96 well dish in triplicate and quantified by plate reader at 565 nm. All transfections were done with 400 nM oligomer by dilution of the specific oligomer with a random oligonucleotide to control for nonspecific oligonucleotide effects.

Fig. 15 is a graph showing antiproliferative/cytopathic effects in Mia Paca II cells by antisense oligonucleotides against the RPA70 gene. Cells were transfected with a matched oligonucleotide (13085) or an oligomer with a one base mismatch

(12781). Methods were identical to those described in figure legend 16.

Fig. 16 is a Northern blot showing suppression of Ribonucleotide Reductase (RR) mRNA by antisense oligomers. Mia Paca II cells were transfected and 24 hours later RR mRNA was measured by Northern Blot (for methods see figure legend 11). All oligomers have a phosphorothioate backbone throughout and are without modification. The lower half of each panel is a EtBr stained gel of the total RNA probed. Oligomer 13704 is a scrambled random control oligomer. RR2410GA targets the variance contained in Mia Paca II cells. Oligomer RR2410AG has two mismatches compared to the genotype of Mia Paca II cells. Oligomers RR1030 and RR1031 are negative control oligomers. They are targeted to a region of RR which is not effective for mRNA down-regulation.

Fig. 17 shows a Northern blot which is a performed similarly to the experiments in Fig. 16. MDA-MB 468 cells were transfected and the level of RR mRNA measured after 24 hours. 13706 is a scrambled random control oligomer. 2410AG targets the two variances contained in the MDA-MB 468 cells. Oligomer 2410GA contains two mismatches relative to the genotype of MDA-MB 468 cells. Both 2410AG and 2410GA are identical to RR2410AG and RR2410GA, respectively.

Fig. 18 shows specific suppression of EPRS mRNA using hybrid oligomers. The sequences at the top provide the structures of the oligonucleotides. The graph at the bottom shows the relative specificity of oligonucleotides.

Fig. 19 is two blots showing specific suppression of EPRS mRNA using hybrid oligomers. A549 cells were transfected with the indicated concentrations of the hybrid oligomers (for structure see text). 14977 targets the two variances contained in A549 cells. 14971 contains two mismatches relative to the genotype

of A549 cells.

Fig. 20 is a graph showing inhibition of mutant *ras* using antisense oligonucleotides specific for the mutant form, based on information available in Schwab et al., 1994, PNAS 91:10460-10464.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Introduction

All normal human cells have two copies of each autosomal chromosome (chromosomes 1 through 22); one copy is inherited from each parent. Each chromosome pair thus contains two alleles for any gene. If a single allele of any gene pair is defective or absent, the surviving allele will continue to produce the encoded gene product. Generally, one allele of a gene pair is sufficient to carry on the normal functions of the cell. (Dominant genetic disorders in which mutations in one allele are sufficient to cause disease are generally those in which the mutation, or gene product harboring the mutation, has a toxic effect on the cell.)

Because humans are genetically heterogeneous, many of the paired alleles of genes of the somatic cells of an individual differ from one another in their gene sequence. Typically both alleles are transcribed and ultimately translated into proteins used by the cell. In most cases, the sequence differences between two allelic forms of a gene in an individual are small, usually differing by only one or a few base differences in sequence. The sequence differences may occur at a single variance site, or may constitute more than one variance site, *i.e.*, two allelic forms in an individual may have more than one sequence variance distinguishing them.

When a cell is heterozygous, *i.e.*, has at least one sequence variance, within the transcribed sequence for a particular gene, each allele may encode a different mRNA, *i.e.*, the mRNAs differ in base sequence. For base changes which are located within coding sequences, the effect of the nucleotide difference depends on whether the base change changes the amino acid which is encoded by the relevant codon. Many base changes do not change the coding sequence because they lie in untranslated regions of the mRNA, outside of the mRNA in introns or intergenic sequences, or in a "wobble" position of a codon which changes the codon, but not

the amino acid it encodes. As a result, the mRNAs encoded by two alleles may translate into the same protein or into forms of the same protein differing by one or more amino acids. An important aspect of the present invention is that many sequence variances that are targets for cancer therapy by the methods described here are not mutations, are not functionally related to cancer, and may not, under normal environmental conditions, induce any function difference between the allelic forms of the gene or protein. Only in the circumstances described in this invention, namely genes that encode essential functions, the presence of variances with a sufficient population frequency, a sufficient frequency of LOH in cancers, do these genes, and the variant sequences within these genes, have utility for the therapy of cancer and other disorders through the discovery of variance-specific inhibitors.

Gene targets for a variance-specific inhibition strategy in this invention satisfy three criteria:

1. The target gene encodes a gene product, *e.g.*, a RNA transcript or protein product essential for the growth or survival of cells.
2. The target gene is located within a chromosome region frequently deleted in cancer cells or cells of a noncancer, proliferative disorder.
3. The target gene exists in two alternative forms in the normal somatic cells of a patient having a cancer or noncancer proliferative disorder.

The allele specific therapy strategy for cancer and noncancer proliferative disorders utilizes the genetic differences between normal cells and neoplastic cells. Thus, the first step in the therapeutic strategy is identifying genes which code for proteins or other factors essential to cell survival and growth that are lost through LOH in tumor cells. Since many genes have been mapped to specific chromosomal regions, this identification can be readily performed by identifying such essential genes which are located in the chromosomal regions characteristically or frequently deleted in

different forms of human cancer or other tumors. Table 2, from the review conducted by Lasko *et al.*, 1991, *Ann. Rev. Genetics* 25:281-314, summarizes results of numerous studies determining loss of heterozygosity in tumors, identifying specific tumor types. A much larger summary of tumor-related LOH is provided in Fig. 5.

Once regions of LOH are identified in the chromosomes of a patient's tumor cells, genes which map to the deleted chromosomal segments and are known to code for gene products essential for cell growth or survival are tested for DNA sequence variances. The identification of a greater number of LOH sites affords a broader selection of target genes coding for essential proteins or other gene products and therefore of sequence variance sites for targeting.

Essential genes which have sequence variants in a population provide a set of target which are advantageous due to the presence of many patients heterozygous for a particular gene, so that the gene will provide a target in cases where the gene has undergone tumor-related LOH.

In accord with the description of target gene categories above, most advantageously a target gene is an essential gene which undergoes LOH in a tumor at a high frequency as described above and which has alternative allelic forms in a population at frequencies as described above. Such genes will provide many potentially treatable patients due to the conjunction of LOH and heterozygosity frequencies.

The most preferred target genes are those essential genes which have both a preferable rate of heterozygosity and a preferable frequency of LOH in a tumor or other proliferative condition in a population of interest. Also preferable is that the gene undergoes LOH in a plurality of different tumors or other conditions.

II. Essential Cellular Function and Essential Genes

As indicated in the Summary above, the invention targets specific allelic forms of essential genes, which are also termed genes essential for cell growth or viability. As used herein the term, "genes which code for a protein essential for the growth or survival or cells" or "genes which code for proteins or factors required for cell viability" or "essential genes" is meant to include those genes that express gene products (*e.g.*, proteins) required for cell survival as well as those genes required for cell growth in actively dividing cell populations. These genes encode proteins which can be involved in any vital cell. An additional factor which applies to genes identified by any of the approaches described above is: a target gene or protein should be encoded by a single locus in man.

A large number of references have identified essential genes which constitute actual or potential targets for allele specific inhibition. The identification of essential genes can be approached in various ways.

1. What are the essential functions each cell must perform to sustain life, and what are the proteins responsible for performing those functions? This is a top down approach for identifying candidate genes whose essential role is then proven experimentally (see below). This approach enables essential genes to be categorized according to the essential cellular process or function which the gene product provides or of which the gene product is a necessary part. Table 1 shows such categories of essential genes and gene functions. In addition, the chromosomal location, where known, and gene product of certain example genes is provided. Thus, the categories of functions shown provide potential targets for the methods of this invention.

2. What genes have been proven essential for cell survival by mutagenesis or gene disruption experiments in cells of other organisms, such as hamster cells, mice,

flies, yeast, bacteria or other organisms? The idea of determining the necessity of specific genes for survival of an organism is well established in simple organisms such as bacteria and yeast. The consequences of gene disruption are easier to assess in these microorganisms that have a haploid genome because the haploid organism contains only one form of a particular single copy gene. A particularly useful category of eukaryotic organisms are the yeasts, especially *Saccharomyces cerevisae.*

3. What are the protein targets of proven mammalian cytostatic and cytotoxic agents such as chemotherapy drugs and poisons?
4. What can be learned from genomics about the genes required for cell survival? This analysis includes identification of the minimal gene set in simple prokaryotes, as well as sequence comparisons across widely divergent species.
5. Experimental testing of gene essentiality. As an example, antisense oligonucleotides can be used to down regulate candidate essential genes (identified by the four approaches listed above) and assess the effects on cell proliferation and survival. Application of an antisense approach to the identification of essential genes was described by Pestov & Lau, *supra.*

Once a gene coding for a protein or factor essential to cell viability is identified, its genomic DNA and cDNA sequences, if not previously established, can be ascertained and sequenced according to standard techniques known to those skilled in the art. See, for example, Sambrook, Fritsch and Maniatis, "Molecular Cloning, A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

Categories of essential genes

Many essential genes function by encoding a gene product which is necessary for maintaining the level of a cellular constituent within the levels required for cell survival or proliferation. The survival and proliferation of cells within the body requires maintaining a state of homeostasis among many different cellular

constituents. These may include, but are not limited to, specific proteins, nucleic acids, carbohydrates, lipids, organic ions, and inorganic ions, or cytoskeletal elements. The loss of homeostasis often results in cell death or apoptosis or inhibition of cell proliferation. Homeostasis in a living cell is dynamic, and programed changes in homeostasis are required through the life cycle of the cell. We have determined that those genes whose products are required for maintaining this homeostasis conducive to cell growth and survival are targets for anti-neoplastic *e.g.*, anti-cancer, inhibitors as described in the methods herein. For example, many genes are involved in synthetic functions, allowing the cells to produce essential cellular constituents including proteins, nucleic acids, carbohydrates, lipids, or organic ions or their components. Other genes are involved in the transport of essential constituents such as proteins, nucleic acids, carbohydrates, lipids, organic ions, or inorganic ions, or their components into the cell or among its internal compartments. Still other genes are involved in the chemical modification of cellular constituents to form other constituents with specific activities. Still other genes are involved in the elimination of specific cellular constituents such as proteins, nucleic acids, carbohydrates, lipids, organic ions, inorganic ions, or their components by metabolic degradation or transport out of the cell. The analysis is preferably carried out using genes which have been shown to be essential in human cells or which are human homologs of genes which are essential in other organisms, preferably other eukaryotic organisms although useful essential data is also provided by prokaryotic essential genes.

A specific example are those genes that are involved in maintaining the amount and fidelity of DNA within a cell. This includes genes commonly considered to be involved in "replication" and other functions; comprising genes involved in the synthesis (polymerization) of DNA sequences from its component elements, creating specific modifications of DNA, ensuring the proper compartmentalization of DNA during cell division (within the nucleus), and eliminating damaged DNA.

This also includes those genes involved in maintaining the amount of nucleosides that are the component elements of DNA by synthesis, salvage, or transport.

Another example are those genes that are involved in maintaining the amount of RNAs within a cell. This includes genes commonly considered to be involved in transcription and other functions; comprising genes required for the synthesis (polymerization) of linear RNA sequences from its component elements, ensuring proper compartmentalization of RNA within the cell, creating specific modification of the linear RNA molecule, and eliminating RNA. This also includes those genes involved in maintaining the amount of nucleosides that are the component elements of RNA by synthesis, salvage, or transport.

Another example are those genes that are involved in maintaining the amount of proteins within a cell. This includes those genes commonly considered to be part of "translation" and other functions;/ comprising genes required for transporting or synthesizing amino acids that are the component elements of proteins, synthesizing specific linear protein sequences from these amino acid elements, creating specific modifications of proteins including by not limited to the addition of specific nucleic acids, carbohydrates, lipids, or inorganic ions to the protein structure, ensuring the proper compartmentalization of synthesized proteins in the cell, and ensuring the proper elimination of proteins from the cell.

Another example are those genes that are involved in maintaining the amount of organic ions within the cell, including but not limited to amino acids, organic acids, fatty acids, nucleosides, and vitamins. This includes those genes that are required for transporting, or synthesizing organic ions, ensuring their proper compartmentalization within the cell, and ensuring proper elimination or degradation of these ions.

Another example are those genes that are involved in maintaining the amount of inorganic ions within the cell. This includes those genes that are required for transporting inorganic ions, including but not limited to O, Na, K, Cl, Fe, P, S, Mn, Mg, Ca, H, PO4 and Zn, ensuring their proper compartmentalization within the cell by binding or transporting these ions, and ensuring proper elimination from the cell.

Another example are those genes that are involved in maintaining the structures and integrity of the cell as described in Example 6 below.

The above groups of genes are shown in Table 1 below, which also points out useful subcategories of genes and lists particular exemplary target genes. This demonstrates that target genes can be grouped according to cellular function to provide classes of essential genes useful for allele specific targeting. Additional target genes can be identified by routing methods, such as those described herein. Confirmation of the essentiality of an additional gene in a specified gene category, and of the occurrence in normal somatic cells of sequence variances of the gene, and of the occurrence of LOH affecting the gene in a neoplastic disorder, establishes that the gene is a target gene potentially useful for identifying allele specific inhibitors and for other aspects of the invention. In addition, as described, target genes are useful in embodiments of certain aspects of the invention, e.g., transplantation and the use of essential or conditionally essential genes even in the absence of LOH.

Table 1

Gene Name	GenBank Accession #
1) Genes Required For Cell Proliferation	

1.1 Genes that regulate cell division

Cyclins, cyclin dependent kinases, regulators and effectors of cyclins and cyclin-dependent kinases	
14-3-3 Protein TAU	X56468
CCNA(G2/Mitotic-Specific Cyclin A)	X51688
CCNB1(G2/Mitotic-Specific Cyclin B1)	M25753
CCND1(G1/S-Specific Cyclin D1)	M73554
CCND2(G1/S-Specific Cyclin D2)	M90813
CCND3(G1/S-Specific Cyclin D3)	M90814
Cell division control protein 16	U18291
Cell division cycle 2, G1 to S and G2 to M	X05360
Cell division cycle 25A	M81933
Cell division cycle 25B	M81935
Cell division cycle 25C	M34065
Cell division cycle 27	U00001
Cell division-associated protein BIMB	D79987
Cyclin A1(G2/Mitotic-Specific Cyclin A1)	U66838
Cyclin C (G1/S-Specific Cyclin C)	M74091
Cyclin G1(G2/Mitotic-Specific Cyclin G)	X77794
Cyclin G2 (G2/Mitotic-Specific Cyclin G)	U47414
Cyclin H	U11791
Cyclin H Assembly	X87843
GSPT1(G1 to S phase transition 1)	X17644
Mitotic MAD2 Protein	U31278
MRNP7	X98263
RANBP1(RAN binding protein 1)	D38076
WEE1	X62048
Cell Division Protein Kinase 4	U79269
CDC28 protein kinase 1	X54941
CDC28 protein kinase 2	X54942
M-Phase inducer phosphatase 2	M81934
M-phase phosphoprotein, mpp6	X98260
PPP1ca(Protein phosphatase 1, catalytic subunit, alpha isoform)	M63960
STM7-LSB	X92493
1.2 Genes that form structures of cell division including the centromere, kinetochore, kinesins, spindle pole body, chromatin assembly factors and their regulators	
CENP-F kinetochore protein	U19769
Centromere autoantigen C	M95724

Centromere protein B (80kD)	X05299
Centromere protein E (312kD)	Z15005
CHC1(Chromosome condensation 1)	X12654
Chromatin assembly factor-in p150 subunit	U20979
Chromatin assembly factor-in p60 subunit	U20980
Chromosome segregation gene homolog CAS	U33286
HMG1(High-mobility group (nonhistone chromosomal) protein 1)	D63874
Minichromosome Maintenance (MCM7)	D28480
Mitotic centromere-associated kinesin	U63743
RMSA1(Regulator of mitotic spindle assembly 1)	L26953
SUPT5h(Chromatin structural protein homolog (SUPT5H))	Y12790

2) Genes Required to Maintain Inorganic Ions and Vitamins at Levels Compatible with Cell Growth or Survival

2.1 Transport of inorganic ions and vitamins across the plasma membrane and intracellular membranes

Active transporters

Uniporters

PMCA1 (Calcium Pump)	U15686
PMCA2 (Calcium Pump)	M97260
PMCA3 (Calcium Pump)	U15689
PMCA4 (Calcium Pump)	M83363
ATP2b1 (Calcium-Transporting ATPase Plasma Membrane)	J04027
ATP2b2 (Calcium-Transporting ATPase Plasma Membrane)	X63575
ATP2b4 (Calcium-Transporting ATPase Plasma Membrane)	M83363
ATP5b (ATP Synthase Beta Chain, Mitochondrial Precursor)	X03559
Chloride Conductance Regulatory Protein ICLN	X91788
H-Erg (Potassium Channel Protein EAG)	U04270
Nuclear Chloride Ion Channel Protein (NCC27)	U93205
SCN1b(Sodium Channel, Voltage-Gated, Type in, Beta Polypeptide)	L16242
Two P-Domain K+ Channel TWIK-1	U33632
VDAC2 (Voltage-Dependent Anion-Selective Channel Protein 2)	L06328

Coupled transporters

Symporters

ATP1b1 (Sodium/Potassium-Transporting ATPase Beta-1 Chain)	X03747

ATP1b2 (Sodium/Potassium-Transporting ATPase Beta-2 Chain)	M81181
Antiporters	
ATPase, Ca++ transporting, plasma membrane 4	M25874
ATPase, Ca++ transporting, plasma membrane 2	L20977
ATPase, Na+/K+ transporting, alpha 1 polypeptide	U16798
ATPase, Na+/K+ transporting, alpha 3 polypeptide	X12910
ATPase, Na+/K+ transporting, beta 1 polypeptide	U16799
ATPase, Na+/K+ transporting, beta 2 polypeptide	U45945
Na+,K+ ATPase, 1 Subunit	
Na+,K+ ATPase, 2 alpha	
Na+,K+ ATPase, 3 beta	U51478
SLC9a1(Solute carrier family 9 (sodium/hydrogen exchanger))	M81768
Solute carrier family 4, anion exchanger, member 1	M27819
Solute carrier family 4, anion exchanger, member 2	U62531
Solute carrier family 9 (sodium/hydrogen exchanger),	X76180
Passive transporters	
MaxiK Potassium Channel Beta Subunit	U25138
Chloride Channel 2	X83378
Chloride Channel Protein (CLCN7)	U88844
TRPC1 (Transient Receptor Potential Channel 1)	X89066
Potassium Channel Kv2.1	L02840
ATP5d(ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit)	X63422
ATP5f1(ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b)	X60221
ATP5o(ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit)	X83218
ETFa(Electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II))	J04058
ETFb(Electron-transfer-flavoprotein, beta polypeptide)	X71129
Nadh-ubiquinone oxidoreductase 13 kd-B subunit	U53468
Nadh-ubiquinone oxidoreductase 39 kD subunit precursor	L04490

NADH-Ubiquinone oxidoreductase 75 kD subunit precursor	X61100
NADH-Ubiquinone oxidoreductase MFWE subunit	X81900
NDUFV2(NADH dehydrogenase (ubiquinone) flavoprotein 2 (24kD))	M22538
Ubiquinol-cytochrome c reductase complex 11 kD	M36647
ATP Synthase Alpha Chain	D14710
NADH dehydrogenase-ubiquinone Fe-S protein 8, 23 kDa subunit	U65579
Vitamin transporters	
Ascorbic Acid (uncloned)	
Folate Binding Protein	AF000380
Folate receptor 1 (adult)	M28099
Nicotinamide (uncloned)	
Pantothenic Acid	X92762
Riboflavin (uncloned)	
SCL19A1 (Solute Carrier Family 19, Member1)	
Solute carrier family 19 (folate transporter), member 1	U19720
Thiamine, B6, B12 (uncloned)	
Metal transporters	
ATP7b (Copper-Transporting ATPase 2)	U03464
Ceruloplasmin (ferroxidase)	M13699
Ceruloplasmin receptor (Copper Transporter)	
Copper Transport Protein HAH1	U70660
Molybdenum, Selenium, other Transporters (uncloned)	
Tranferrin Receptor (Iron Transporter)	X01060
Zinc Transporter (uncloned)	
Soluble inorganic ion transporters	
Insoluble inorganic ion transporters	
Transporters of other essential small molecules	
Mitochondrial Import Receptor Subunit TOM20	D13641
2.2 Regulators of transport	
Sensors of ion levels	
3) Genes Required to Maintain Organic Compounds at Levels Compatible with Cell Growth or Survival	
3.1 Transporters of organic compounds	
Carbohydrate Transport	
Sugar Transport	
Glucose Transport	

GLUT1	GDB:120627
GLUT2	J03810
GLUT3	M20681
GLUT4	M20747
GLUT5	M55531
GLUT6	M95549
Solute carrier family 5 (sodium/glucose cotransporter)	M95549
Solute carrier family 2 (facilitated glucose transporter), member 2	J03810
Solute carrier family 2 (facilitated glucose transporter) member 5	M55531
Amino acid transport	
Solute carrier family 3 member 1	L11696
System b,(Na+ independent)	
System y,(Na+ independent)	
ATRC1(Catioinc)	OMIM 104615
LEUT(Leucine Transporter)	OMIM 151310
SLC1A1(Solute Carrier Family 1, Member 1)	OMIM 133550
Lipid or lipoprotein transport	
Nucleoside transport	
Other organic compounds transport	
Solute carrier family 16 (monocarboxylic acid transporters)	L31801
3.2 Genes required for maintenance of organic compounds at levels required for cell growth or survival	
Carbohydrate metabolism, including anabolism and catabolism	
ACO1(Aconitase 1)	
ACO2(Aconitase 2, mitochondrial)	U80040
Acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain	M26393
Acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	M16827
Acyl-Coenzyme A dehydrogenase, long chain	M74096
Acyl-Coenzyme A dehydrogenase, very long chain	D43682
aKGD (alpha ketoglutaratedehydrogenase)	
ALD-a (Aldolase)	M11560
ALD-b (Aldolase)	K01177
ALD-c (Aldolase)	M21191
CS (Citrate Synthetase)	OMIM 118950
Dihydrolipoamide S-succinyltransferase	L37418
DLAT(Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex))	AF001437
DLD(Dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex))	J03490
E1k (Oxoglutarate dehydrogenase)	D10523

E2k (Dihydrolipoamide S-succinyltransferase)	D16373
E3 (Dihydrolipoyl Dehydrogenase)	SEG_HUMDHL
ENO1(Enolase 1,alpha)	M14328
ENO2(Enolase 2)	X51956
ENO3(Enolase 3)	X55976
Enolase 2, (gamma, neuronal)	M22349
Enolase 3, (beta, muscle)	X16504
FH(Fumarate hydratase)	M15502
G3PDH (Glyceraldehyde-3-Phosphate Dehydrogenase)	M17851
G6PD (Glucose-6-Phosphate Dehydrogenase)	
Glucose-6-phosphate dehydrogenase	X03674
HK1 (Hexokinase 1)	M75126
HK2 (Hexokinase 2)	S70035
HK3 (Hexokinase 3)	U51333
IDH1(Isocitrate dehydrogenase 1 (NADP+), soluble)	OMIM 147700
IDH2(Isocitrate dehydrogenase 2 (NADP+), mitochondrial)	X69433
MDH1(Malate dehydrogenase 1, NAD (soluble))	D55654
MDH2(Malate dehydrogenase 1, NAD (mitochondrial))	OMIM 154100
NAD(H)-specific isocitrate dehydrogenase alpha subunit	U07681
Oxoglutarate dehydrogenase (lipoamide)	D10523
PDHB (Pyruvate Dehydrogenase)	J03576
PDHB(Pyruvate dehydrogenase (lipoamide) beta)	M34479
PDK4 (Pyruvate dehydrogenase kinase, isoenzyme 4)	U54617
PFKL(Phosphofructokinase)	M10036
PGI (Phosphoglucoisomerase)	OMIM 172400
PGKa (Phosphoglyceromutase)	Y00572
PGKb (Phosphoglyceromutase)	K03201
PGM1 (Phosphoglyceromutase)	M83088
PGM2 (Phosphoglyceromutase)	OMIM 172000
PGM3 (Phosphoglyceromutase)	OMIM 172100
PGM4 (Phosphoglyceromutase)	OMIM 172110
Phosphofructokinase, muscle	U24183
Phosphoglucomutase 1	M83088
Phosphoglycerate kinase 1	V00572
PK1 (Pyruvate Kinase)	M15465
PK2 (Pyruvate Kinase)	OMIM 179040
PK3 (Pyruvate Kinase)	M23725
Pyruvate dehydrogenase kinase isoenzyme 2 (PDK2)	L42451
Pyruvate kinase, liver	D10326
Pyruvate kinase, muscle	M23725
SDH1(Succinate dehydrogenase, iron sulphur (Ip) subunit)	D10245
SDH2(Succinate dehydrogenase 2, flavoprotein (Fp) subunit)	D30648
TKT(Transketolase (Wernicke-Korsakoff syndrome))	L12711
TPI (Trisephosphate Isomerase)	M10036

Amino Acid biosynthesis and processing	
Asparagine Synthetase	SEG_HUMASN
Aminoacylase-1	L07548
Aminoacylase-2	S67156
Fatty acid biosynthesis and processing	
ACAC (Acetyl CoA Carboxylase Beta)	U19822
ACAC (Acetyl CoA Carboxylase)	U12778
ACADSB(Acyl-coA dehydrogenase)	U12778
Mevalonate kinase	M88468
Phosphomevalonate kinase	L77213
Alcohol biosynthesis and processing	
Other organic compounds biosynthesis and processing	
Aspartoacylase	S67156
Ornithine decarboxylase 1	M16650
3.3 Genes required for catabolism, degradation and elimination of organic compounds	
Carbohydrate and Sugar Catabolism	
Amino acid Degradation	
Lipid or lipoprotein Degradation	
Short-acyl-CoA dehydrogenase	M26393
Medium acyl-CoA dehydrogenase	S75214
Long acyl-CoA dehydrogenase	M74096
Isovalveryl CoA dehydrogenase	M34192
2-methyl branched chain	
Nucleoside Degradation	
Adenosine Deaminase	K00509
Purine-nucleoside phosphorylase	K02574
Guanine Deaminase	
Xanthine Oxidase	D11456
Degradation of other organic compounds	
3.4 Genes Required to Modify Polypeptides, Lipids or Sugars by Addition, Removal or Modification of Chemical Groups to Form Compounds Necessary for Cell Growth or Survival	
Addition, removal or modification of sugar groups	
Glycosyltransferases	
Glycosylases	
ITM1 (Integral Transmembrane Protein)	L38961
GFPT (Glutamine-Fructose-6-Phosphate Transaminase)	M90516
Heparan	U36601
Polypeptide N-Acetyltransferase	U41514
Addition, removal or modification of methyl or other alkylgroups	
Acetyltransferase	
ACAA(Acetyl-Coenzyme A acyltransferase)	X12966
Lysophosphatidic acid acyltransferase-alpha	U56417

Lysophosphatidic acid acyltransferase-beta	U56418
Farnesyltransferase	
FNTa (Farnesyltransferase Alpha Subunit)	L00634
FNTb (Farnesyltransferase Beta Subunit)	L00635
Myristoylation	
NMT1 (N-myristoyltransferase)	
Addition, removal or modification of sulfhydryl groups	
Addition, removal or modification of phosphate groups	
Calcineurin A	S46622
Calcineurin B	M30773
Calreticulin Precursor	M84739
Phosphatase 2b	M29551
PPP3ca(Protein phosphatase 3 , catalytic subunit)	J05480
SNK Interacting 2-28(Calcineurin B Subunit)	U83236
Protein Kinase C	
PRKCA(Protein kinase C, alpha)	X52479
PRKCB1(Protein kinase C, beta 1)	X06318
PRKCD(Protein kinase C, delta)	L07861
PRKCM(Protein kinase C, mu)	X75756
PRKCQ(Protein kinase C-theta)	L01087
PRKCSH(Protein kinase C substrate 80K-H)	J03075
Addition, removal or modification of lipid groups	
Geranylgeranyl	
Geranylgeranyltransferase (Type I Beta)	L25441
GGTB (Geranylgeranyltransferase)	Y08201
Geranylgeranyltransferase (Type II Beta-Subunit)	X98001
3.5 Genes required for regulation of levels of organic ions	
Gdp Dissociation Inhibitors	
GDI Alpha (RAB GDP Dissociation Inhibitor Alpha)	D45021
Rab Gdp (RAB GDP Dissociation Inhibitor Alpha)	D13988

4) Genes Required to Maintain Cellular Proteins at Levels Compatible with Cell Growth or Survival

Polypeptide precursor biosynthesis	
Amino acid biosynthesis and modification	
GOT(Glutamic-oxaloacetic transaminase 2)	M22632
GOT1(Glutamic-oxaloacetic transaminase 1)	M37400
PYCS(Pyrroline-5-carboxylate synthetase)	X94453
Tyrosine aminotransferase	X52520
Polypeptide precursor elimination	
Synthesis of components for polypeptide polymerization	
AARS	D32050
CARS	L06845
DARS	

EPRS	X54326
FARS	
GARS	U09510
HARS	X05345
IARS	D28473
KARS	OMIM 601421
LARS	OMIM 151350
MARS	X94754
NARS	M27396
QARS	X54326
RARS	S80343
SARS	
TARS	M63180
VARS	X59303
WRS	M61715
YARS	
Polypeptide polymerization	
Ribosome Subunits	
Ribosomal Protein L11	X79234
Ribosomal Protein L12	L06505
Ribosomal Protein L17	X52839
Ribosomal Protein L18	L11566
Ribosomal Protein L18a	X80822
Ribosomal Protein L19	X63527
Ribosomal Protein L21	U14967
Ribosomal Protein L22	L21756
Ribosomal Protein L23	X53777
Ribosomal Protein L23a	U43701
Ribosomal Protein L25	
Ribosomal Protein L26	
Ribosomal Protein L27	L19527
Ribosomal Protein L27a	U14968
Ribosomal Protein L28	U14969
Ribosomal Protein L29	U10248
Ribosomal Protein L30	OMIM 180467
Ribosomal Protein L31	
Ribosomal Protein L32	X03342
Ribosomal Protein L35	U12465
Ribosomal Protein L35a	X52966
Ribosomal Protein L36a	OMIM 180469
Ribosomal Protein L39	U57846
Ribosomal Protein L4	L20868
Ribosomal Protein L41	
Ribosomal Protein L44	

Ribosomal Protein L6	X69391
Ribosomal Protein L7	L16558
Ribosomal Protein L7a	X52138
Ribosomal Protein L8	Z28407
Ribosomal Protein L9	U09953
Ribosomal Protein P1	M17886
Ribosomal Protein S10	U14972
Ribosomal Protein S11	X06617
Ribosomal Protein S13	L01124
Ribosomal Protein S14	
Ribosomal Protein S15	J02984
Ribosomal Protein S15A	X84407
Ribosomal Protein S16	M60854
Ribosomal Protein S17	M13932
Ribosomal Protein S17A	OMIM 180461
Ribosomal Protein S17B	OMIM 180462
Ribosomal Protein S18	L06432
Ribosomal Protein S20	
Ribosomal Protein S20A	OMIM 180463
Ribosomal Protein S20B	OMIM 180464
Ribosomal Protein S21	L04483
Ribosomal Protein S23	D14530
Ribosomal Protein S25	M64716
Ribosomal Protein S26	X69654
Ribosomal Protein S28	U58682
Ribosomal Protein S29	L31610
Ribosomal Protein S3	U14990
Ribosomal Protein S3A	OMIM 180478
Ribosomal Protein S4	
Ribosomal Protein S4X	M58458
Ribosomal Protein S4Y	M58459
Ribosomal Protein S5	U14970
Ribosomal Protein S6	J03537
Ribosomal Protein S7	M77233
Ribosomal Protein S8	OMIM 600357
Ribosomal Protein S9	U14971
Initiation of polypeptide polymerization	
eIF-2 (Eukaryotic initiation factor)	L19161
eIF-2-associated p67(Eukaryotic initiation factor)	U13261
eIF-2A(Eukaryotic initiation factor)	J02645
eIF-2Alpha(Eukaryotic initiation factor)	U26032
eIF-2B(Eukaryotic initiation factor)	U23028
eIF-2B-Gamma(Eukaryotic initiation factor)	L40395
eIF-2Beta(Eukaryotic initiation factor)	M29536

eIF-3 p110(Eukaryotic initiation factor)	U46025
eIF-3 p36(Eukaryotic initiation factor)	U39067
eIF-4A(Eukaryotic initiation factor)	D21853
eIF-4C(Eukaryotic initiation factor)	L18960
eIF-4E(Eukaryotic initiation factor)	M15353
eIF-4Gamma(Eukaryotic initiation factor)	Z34918
eIF-5(Eukaryotic initiation factor)	U49436
eIF-5A	
Polypeptide elongation	
Eukaryotic peptide chain release factor subunit 1	X81625
P97(Eukaryotic initiation factor)	U73824
eEF1A2(Eukaryotic elongation factor)	X70940
eEF1D(Eukaryotic elongation factor)	Z21507
eEF2(Eukaryotic elongation factor)	X54166
eIF4A2 (Eukaryotic initiation factor)	D30655
KIAA0031(Elongation factor 2)	D21163
KIAA0219(Putative translational activator C18G6.05C)	D86973
Factor 1-Alpha 2(Eukaryotic translation elongation factor 1 alpha 2)	D30655
Termination of polypeptide polymerization	
Polypeptide folding	
Cis-Trans Isomerase	M80254
DNAj Protein Homolog 1	X62421
DNAj Protein Homolog 2	D13388
DNAJ Protein homolog HSJ1	X63368
Chaperone proteins	
T-Complex	
Aspartylglucosaminidase	X55330
T-Complex 1, Alpha	S70154
T-Complex 1, Epsilon	D43950
T-Complex 1, Gamma	X74801
T-Complex 1, Theta	D13627
T-Complex 1, Zeta	M94083
Polypeptide Degradation	
Proteasome components and proteinases	
26S Protease regulatory subunit 4	L02426
Alpha-2-Macroglobulin	M11313
Calpain 1, Large	X04366
CLPP(ATP-Dependent CLP protease proteolytic subunit)	Z50853
KIAA0123 (Mitochondrial processing peptidase alpha subunit)	D50913
MMP7	X07819
Proteasome Beta 6	D29012
Proteasome Beta 7	D38048
Proteasome C13	U17496

Proteasome C2	D00759
Proteasome C7-1	D26599
Proteasome inhibitor hPI31 subunit	D88378
Proteasome P112	D44466
Proteasome P27	AB003177
Proteasome P55	AB003103
Ubiquitin System	
Enzyme E2-17 Kd(Cyclin-selective ubiquitin carrier protein)	U73379
ISOT-3(Ubiquitin carboxyl-terminal hydrolase T)	U75362
ORF (Ubiquitin carboxyl-terminal hydrolase 14)	M68864
PGP(Ubiquitin carboxyl-terminal hydrolase isozyme L1)	X04741
UBA52(Ubiquitin A-52 residue ribosomal protein fusion product 1)	S79522
Ubiquitin carboxyl-terminal hydrolase 3	D80012
Ubiquitin carboxyl-terminal hydrolase isozyme L3	M30496
Ubiquitin carboxyl-terminal hydrolase T	X91349
Ubiquitin carrier protein (E2-EPF)	M91670
Ubiquitin fusion-degradation protein (UFD1L)	U64444
Ubiquitin Hydrolase	X98296
Ubiquitin-conjugating enzyme E2I	U45328
Polypeptide Transport	
SEC23(Protein transport protein SEC23)	X97065
SEC23A(Protein transport protein SEC23)	X97064
SEC7(Protein transport protein SEC7)	X99688
SEC61 (Beta Subunit)	L25085
Lipoprotein Transport	
LDLR (LDL receptor)	

5) Genes Required to maintain Cellular Nucleotides at Levels Compatible with Cell Growth or Survival

Genes Required to Maintain Cellular DNA with Fidelity and at Levels Compatible with Cell Growth or Survival

DNA Precursor Biosynthesis

Adenylate Kinase-2	U39945
Adenylosuccinate synthetase	X66503
Adenylosuccinate Lyase	X65867
ADPRT (ADP-Ribosyltransferase)	M32721
ADSL (Adenylosuccinate lyase/AMP synthetase)	X65867
ADSS (Adenylosuccinate Synthetase)	X66503
CAD PROTEIN	D78586
CTP Synthetase	
CTPS(CTP synthetase)	X52142
Cytidine Triphosphate Synthetase	
GARS (Phosphoribosylglycinamide synthetase)	D32051

GART (Phosphoribosylglycinamide formyltransferase)	
GART(Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase)	X54199
GMP Synthetase	U10860
IMP Cyclohydrolase	U37436
IMP dehydrogenase	L19709
IMPDH1(IMP (inosine monophosphate) dehydrogenase 1)	J05272
IMPDH2(IMP (inosine monophosphate) dehydrogenase 2)	J04208
Phosphoribosyl diphosphotransferase	
Phosphoribosylaminoimidazolecarboxamide formyltransferase	
Phosphoribosylformylglycinamide synthetase	M32082
Phosphoribosylglycinamide carboxylase	
Phosphoribosylglycinamide-succinocarboxamide synthetase	
PPAT (Amidophoribosyltransferase)	
PPAT(Phosphoribosyl pyrophosphate amidotransferase)	U00238
Ribonucleoside-diphosphate reductase M1 chain	X59543
Ribonucleoside-diphosphate reductase M2 chain	X59618
Thymidine Kinase	K02581
Thymidylate Synthase	X02308
UMK(Uridine kinase)	D78335
UMPK (Uridine monophosphate kinase)	OMIM 191710
UMPS(Uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase))	J03626
Uridine Phosphorylase	X90858
DNA Precursor Elimination	
DNA Replication	
Origin Recognition	
Origin Recognition Complex	
ORC1	U40152
ORC2	U27459
ORC3	
ORC4	
ORC5	OMIM 602331
ORC6	
ORC Regulators	
CDC6	AA830372
CDC7	AFO15592
CDC18	AF022109
DNA Polymerization	
DNA Polymerases	
Adprt (NAD(+) ADP- Ribosyltransferase)	M18112
DNA Polymerase Alpha-Subunit	X06745
DNA Polymerase Delta	U21090

POLa(DNA Polymerase Alpha/Primase Associated Subunit)	L24559
POLb(DNA Polymerase Beta Subunit)	D29013
POLd1(Polymerase (DNA directed), Delta 1, Catalytic Subunit)	M81735
POLd2(Polymerase (DNA directed), Delta 2)	U21090
POLE(Polymerase (DNA directed))	OMIM 174762
POLg (DNA Polymerase Gamma Subunit)	X98093
Terminal Transferase (DNA Nucleotidylexotransferase)	M11722
Accessory factors for DNA Polymerization	
Activator 1 36 Kd	L07540
CDC46 (DNA Replication Licensing Factor)	X74795
CDC47 (DNA Replication Licensing Factor CDC47)	D55716
DNA Topoisomerase III	U43431
DRAP1 (DNA Replication Licensing Factor MCM3)	U41843
KIAA0030 Gene (Cell Division Control Protein 19)	X67334
KIAA0083 Gene (DNA Replication Helicase DNA2)	D42046
MCM3 (DNA Replication Licensing Factor MCM3)	D38073
PCNA (Proliferating Cell Nuclear Antigen)	J04718
PRIM1 (DNA Primase 49 kD Subunit)	X74330
PRIM2 (DNA Primase)	X74331
PRIM2a (DNA Primase 58 kD Subunit)	X74331
PRIM2b (DNA Primase)	OMIM 600741
RECa (Replication Protein A 14 kD Subunit)	L07493
RFC1 (Replication Factor C (activator 1) 1)	L14922
RFC2 (Replication Factor C 2)	M87338
RFC3 (Replication Factor C (activator 1) 3)	L07541
RFC4 (Replication Factor C, 37-kD subunit)	M87339
RFC5 (Replication Factor C)	OMIM 600407
RPA1 (Replication protein A1 (70kD))	M63488
RPA2 (Replication protein A2 (32kD))	J05249
RPA3 (Replication protein A3 (14kD))	L07493
TOP1 (DNA Topoisomerase I)	J03250
TOP2a (Topoisomerase (DNA) II Alpha (170kD))	J04088
TOP2b (Topoisomerase (DNA) II Beta (180kD))	U54831
DNA Helicases	
CHL1(CHL1-Related Helicase)	U33833
DNA Helicase II	M30938
Mi-2(Chromodomain-Helicase- DNA-Binding Protein CHD-1)	X86691
RECQL (ATP-Dependent DNA Helicase Q1)	L36140
Smbp2 (DNA-Binding Protein SMUBP-2)	L14754
DNA Packaging Proteins	
Histones	
H1(0) (Histone H5A)	X03473
Histone H1d	X57129
Histone H1x	D64142

Histone H2a.1	U90551
Histone H2a.2	L19779
Histone H2b.1	M60756
Histone H4	X60486
SLBP (Histone Hairpin-Binding Protein)	Z71188

DNA Degradation

DNA Repair

Genes Required to Maintain Cellular RNA at Levels Compatible with Cell Growth or Survival

RNA Precursor Biosynthesis

RNA Precursor Elimination

RNA Polymerization

Initiation of polymerization

TATA-binding Complex

Small Nuclear RNA-Activating Complex, Polypeptide 1, 43KD (SNAPC1)	Z47542
Small Nuclear RNA-Activating Complex, Polypeptide 2, (SNAPC2)	
Small Nuclear RNA_Activating Complex, Polypeptide 3, 50KD (SNAPC3)	U71300
TAF2D(TBP-associated factor)	U78525
TAFII100(TBP-associated factor)	X95525
TAFII130(TBP-associated factor)	U75308
TAFII20(TBP-associated factor)	X84002
TAFII250(TBP-associated factor)	D90359
TAFII28(TBP-associated factor)	X83928
TAFII30(TBP-associated factor)	U13991
TAFII32(TBP-associated factor)	U21858
TAFII40(TBP-associated factor)	
TAFII55(TBP-associated factor)	U18062
TAFII80(TBP-associated factor)	U31659
TBP(TATA Binding Protein)	M55654
TMF1 (TATA Element Modulatory Factor 1)	

Polymerization

RPB 7.0	U52427
RPB 7.6	
RPB 17	
RPB 14.4	

RNA Polymerase I subunits

RNA polymerase I subunit hRPA39	AF008442

RNA Polymerase II subunits

13.6 Kd Polypeptide (DNA-Directed RNA Polymerase II 13.6 kD Polypeptide)	L37127
POLR2C(RNA polymerase II, polypeptide C (33kD))	J05448

Polypeptide A (220kd)	X63564
RNA Polymerase II 23k	J04965
RNA polymerase II holoenzyme component (SRB7)	U46837
RNA polymerase II subunit (hsRPB10)	U37690
RNA polymerase II subunit (hsRPB8)	U37689
RNA polymerase II subunit hsRPB4	U85510
RNA polymerase II subunit hsRPB7	U20659
RNA Polymerase II Subunit(DNA- Directed RNA Polymerases I, II, and III 7.3 kD polypeptide)	Z47727
TCEB1L(Transcription elongation factor B (SIII), polypeptide 1-like)	Z47087
RNA Polymerase III subunits	
RNA polymerase III subunit (RPC39)	U93869
RNA polymerase III subunit (RPC62)	U93867
RNA Elongation	
Elongation Factor 1-Beta	X60489
Elongation Factor S-II	M81601
Elongation	
TCEA (110kD)	OMIM 601425
TCEB1	L34587
TCEB (18kD)	
TCEB1L	
TCEB3	L47345
TCEC (15kDa)	
TFIIS (Transcription Elongation Factor IIS)	601425
E2F1 (E2F Transcription Factor)	M96577
TFAP2A (Transcription Factor A2 Alpha)	X95694
TFCP2 (Transcription Factor CP2)	U01965
TFC12 (Transcription Factor 12)	M65209
PRKDC (Protein Kinase, DNA activated catalytic subunit)	U47077
Termination of RNA polymerization	
Factors that regulate RNA polymerization	
General factors	
SUPT6H	U46691
TFIIA gamma subunit	U14193
TFIIA delta	
TFIIB related factor hBRF (HBRF)	U75276
TFIIE Alpha Subunit	X63468
TFIIE Beta Subunit	X63469
TFIIF, Beta Subunit	X16901
GTF2F1 (TFIIF)	X64037
GTF2F2 (TFIIF)	X16901
General Transcription Factor IIIA	U20272
TFIIH(52 kD subunit of transcription factor)	Y07595

TFIIH(p89)	
TFIIH(p80)	
TFIIH(p62)	U07595
TFIIH(p44)	OMIM 601748
TFIIH(p34)	OMIM 601750
Transcription Factor IIf(General transcription factor IIF, polypeptide 1 (74kD subunit))	X64037
Specific factors required for polymerization of essential genes	
BTF 62 kDSubunit (Basic transcription factor 62 kD subunit)	M95809
CAMP-dependent transcription factor ATF-4	M86842
CCAAT box-binding transcription factor 1	X92857
CRM1(Negative regulator CRM1)	Y08614
Cyclic-AMP-dependent transcription factor ATF-1	X55544
GABPA(GA-binding protein transcription factor, alpha subunit (60kD))	U13044
ISGF-3(Signal transducer and activator of transcription 1-alpha/beta)	M97935
NFIX(Nuclear factor I/X (CCAAT-binding transcription factor))	L31881
NFYA(Nuclear transcription factor Y, alpha)	M59079
NTF97(Nuclear factor p97)	L38951
Nuclear factor I-B2 (NFIB2)	U85193
Nuclear factor NF45	U10323
Nuclear factor NF90	U10324
POU2F1(POU domain, class 2, transcription factor 1)	X13403
Sp2 transcription factor	M97190
TCF12(Transcription factor 12 (HTF4, helix-loop-helix transcription factors 4))	M83233
TCF3(Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47))	M31523
TCF6L1(Transcription factor 6-like 1)	M62810
TF P65(Transcription factor p65)	L19067
TFCOUP2(Transcription factor COUP 2 (a.k.a. ARP1))	X91504
Transcription factor IL-4 Stat	U16031
Transcription Factor S-II (Transcription factor S-II-related protein)	D50495
Transcription factor Stat5b	U48730
Transcription Factor	L06633
Transcription factor (CBFB)	L20298
RNA Processing Factors	
RNA splicing and other processing factors	
9G8 Splicing Factor (Pre-mRNA Splicing factor SRP20)	L22253
CC1.3(Splicing factor (CC1.3))	L10910
HnRNP F protein	L28010
HNRPA2B1(Heterogeneous nuclear ribonucleoproteins A2/B1)	M29065
HNRPG(Heterogeneous nuclear ribonucleoprotein G)	Z23064

HNRPK(Heterogeneous nuclear ribonucleoprotein K)	S74678
Pre-mRNA splicing factor helicase	D50487
Pre-mRNA splicing factor SF2, P33 subunit	M69040
Pre-mRNA splicing factor SRP20	L10838
Pre-mRNA splicing factor SRP75	L14076
PRP4(Serine/threonine-protein kinase PRP4)	U48736
PTB-Associated Splicing Factor	X16850
Ribonucleoprotein A'	X06347
Ribonucleoprotein A1	X13482
Ribonucleoprotein C1/C2	M15841
RNP Protein, L (Heterogeneous nuclear ribonucleoprotein L)	X16135
RNP-Specific C(U1 small nuclear ribonucleoprotein C)	X12517
SAP 145(Spliceosome associated protein)	U41371
SAP 61(Splicesomal protein)	U08815
SC35(Splicing factor)	L37368
SF3a120	X85237
SFRS2(Splicing factor, arginine/serine-rich 2)	M90104
SFRS5(Splicing factor, arginine/serine-rich 5)	AF020307
SFRS7(Splicing factor, arginine/serine-rich 7)	L41887
Small nuclear ribonucleoprotein SM D1	J03798
SnRNP core protein Sm D2	U15008
SnRNP core protein Sm D3	U15009
SNRP70(U1 snRNP 70K protein)	M22636
SNRPB(Small nuclear ribonucleoprotein polypeptides B and B1)	J04564
SNRPE(Small nuclear ribonucleoprotein polypeptide E)	M37716
SNRPN(Small nuclear ribonucleoprotein polypeptide N)	U41303
Splicing factor SF3a120	X85238
Splicing factor U2AF 35 kD subunit	M96982
Splicing factor U2AF 65 kD subunit	X64044
SRP30C(Pre-mRNA splicing factor SF2, p33 subunit)	U30825
SRP55-2(Pre-mRNA splicing factor SRP75)	U30828
Transcription factor BTEB	D31716
Transcription initiation factor TFIID 250 kD subunit	D90359
RNA polyadenylation and cleavage	
Cleavage and polyadenylation specificity factor	U37012
Cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kD	L02547
Cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kD	U15782
HNRNP Methyltransferase	D66904
PABPL1(Poly(A)-binding protein-like 1)	Y00345
Pap mRNA(Poly(A) Polymerase)	X76770
RNA unwinding	
RNA Helicase	

GU Protein (ATP-Dependent RNA helicase dead)	U41387
KIAA0224 Gene(Putative ATP-dependent RNA helicase)	D86977
RNA Helicase A	L13848
RNA Helicase P110	U50553
Ste13(Nuclear RNA Helicase)	U90426

RNA Degradation

RNA modification

RNA Transport

6) Genes Required to Maintain Integrity and Function of Cellular and Subcellular Structures

6.1 Genes Required to Move Proteins, Small Particles, and Other Ligands Across Membranes to Maintain their Concentration at Levels Compatible with Cell Growth or Survival

Genes required to form coated pits and vesicles

Clathrins

AP47(Clathrin Coat Assembly AP47)	D38293
AP50(Clathrin Coat Assembly Protein AP50)	U36188
Cell Surface Protein (Clathrin Heavy Polypeptide-Like Protein)	X83545
Cltb(Clathrin Light Chain B)	M20470
Cltc (Clathrin Heavy Chain)	U41763

6.2 Genes Required to Transmit Signals within Cells at Levels Compatible with Cell Growth or Survival

Genes required to transmit signals from membranes

Adenylate Cyclase

Adenylate Cyclase	D63481
Adenylate Cyclase, II	X74210
Adenylate Cyclase,IV	D25538

Genes required to transmit signals within cellular compartments

6.3 Genes Required to Maintain Cellular Energy Stores at Levels Compatible with Cell Growth or Survival

Genes required to Produce ATP from catabolism of sugar

Genes required for glycolysis (anaerobic and aerobic)

Genes required for oxidative phosphorylation

Complex I

MTND1 (Subunit ND1)	OMIM 51600
MTND2 (Subunit ND2)	OMIM 51601
MTND3 (Subunit ND3)	OMIM 51602
MTND4 (Subunit ND4)	OMIM 51603
MTND4L (Subunit ND4L)	OMIM 51604
MTND5 (Subunit ND5)	OMIM 51605
MTND6 (Subunit ND6)	OMIM 51606

Complex II

Complex III

Cytochrome b subunit

Complex IV

CO1 (Cytochrome c Oxidase Subunit I)	OMIM 516030
CO2 (Cytochrome c Oxidase Subunit 2)	AF035429
CO3 (Cytochrome c Oxidase Subunit 3)	
Complex V	
ATP Synthase Subunit ATPase 6	OMIM 516060
6.4 Genes Required to Transport or Dock Vesicles, Polypeptides or Other Solutes Moving Between Cellular Compartments at Rates and Levels Compatible with Cell Growth or Survival	
Transport to, from or within the cytoplasm	
Kinesins	
Kinesin Heavy Chain	X65873
Kinesin Light Chain	L04733
Syntaxin	
Syntaxin 1a	L37792
Syntaxin 1b	U07158
Syntaxin 3	U32315
Syntaxin 5a	U26648
Syntaxin 7	U77942
Transport to, from or within the endoplasmic reticulum	
CANX (Calnexin)	M94859
ER Lumen Protein 1	M88458
ER Lumen Protein 2	X55885
Ribophorin I	Y00281
Ribophorin II	Y00282
Signal recognition particle receptor	X06272
SRP Protein	U20998
TIM17 preprotein translocase	X97544
Transport to, from or within the Golgi apparatus	
Golgin-245	U31906
TGN46 (Trans-Golgi Network Integral Membrane Protein TGN38 Precursor)	X94333
Transport to, from or within the other membrane bound compartments	
Beta-Cop	X82103
Coatomer Beta' Subunit	X70476
Coatomer Delta Subunit	X81198
Gp36b Glycoprotein (Vesicular integral-membrane protein VIP36 precursor)	U10362
Homologue of yeast sec7	M85169
Protein transport protein SEC13 (Chromosome 3p25)	L09260
SEC14 (S. Cerevisiae)	D67029
Synaptic vesicle membrane protein VAT-1	U18009
Synaptobrevin-3	U64520
Synaptotagmin I	M55047
Transmembrane(COP-coated vesicle membrane protein p24 precursor)	X92098

Vacuolar-Type (Clathrin-coated vesicle/synaptic vesicle proton pump 116 kd subunit)	Z71460
Transport to, from or within the nucleus	
Nuclear membrane constituents	
140 kD Nucleolar phosphoprotein	D21262
Autoantigen p542	L38696
Export protein Rae1 (RAE1)	U84720
Heterogeneous nuclear ribonucleoprotein A1	X79536
Nuclear pore complex protein hnup153	Z25535
Nuclear pore complex protein NUP214	D14689
Nuclear pore glycoprotein p62	X58521
Nuclear Transport Factor 2	X07315
Nucleoporin 98 (NUP98)	U41815
NUP88	Y08612
Ribonucleoprotein A	M29063
Ribonucleoprotein B"	U23803
Nuclear envelope & pore constituents	
Karyopherin	
Importin Alpha Subunit	D89618
TRN (Transportin)	U70322
6.5 Genes Required to Maintain Cell Shape and Motility at Levels Compatible with Cell Growth or Survival	
Cell structure genes (Cytoskeleton)	
Actin	X04098
Beta-Centractin	X82207
Capping Protein Alpha	U03851
CFL1 (Cofilin, Non-Muscle Isoform)	X95404
Desmin	J03191
Dystrophin	U26743
Gelsolin	X04412
hOGG1(Myosin Light Chain Kinase)	AB000410
IC Heavy Chain	U31089
Itga2 (Integrin, Alpha 2 (CD49B, alpha 2 Subunit of VLA-2 receptor))	X17033
Itga3 (Integrin Alpha-3 Precursor)	M59911
Keratin 19	Y00503
Keratin, Type II	J00269
Lamin A	M13451
LBR(Lamin B Receptor)	L25931
Light Chain Alkali	M22920
MacMarcks mRNA	X70326
MAP1a (Microtubule-Associated Protein 1A)	U14577
MAP2(Microtubule-Associated Protein 2)	U01828
MEG1(Protein-Tyrosine Phosphatase MEG1)	X79510

Microtubule-Associated Protein TAU	J03778
Suppressor Of Tubulin STU2	X92474
TUBg (Tubulin Gamma Chain)	M61764
Tubulin Alpha-4 Chain	X06956
USH1b (Myosin II Heavy Chain)	U39226
Villin	X12901
Villin 2 (Ezrin)	J05021
Genes required for cell motility	
Actin genes	
Actin Depolymerizing	S65738
Capping (Actin Filament)	M94345
Myosin genes	
MYH9(Myosin, Heavy Polypeptide 9, Non-Muscle)	M31013
MYL5(Myosin Regulatory Light Chain 2)	L03785
Myosin Heavy Chain 95F	U90236
Myosin Heavy Chain IB	D63476
Myosin IB	U14391
Sh3p17(Myosin IC Heavy Chain)	U61166
Sh3p18(Myosin IC Heavy Chain)	U61167
KIAA0059(Dematin:Actin-Bundling Protein)	D31883
TTN (Titin:Myosin Light Chain Kinase)	X69490
6.6 Genes Required to Eliminate, Transform, Sequester or Otherwise Regulate Levels of Endogenous Cellular Toxins or Waste Substances at Levels Compatible with Cell Growth or Survival	
Organelles that transform or sequester toxic or waste substances	
Vacuoles	
ATP6c(Vacuolar H+ ATPase proton channel subunit)	M62762
Lysosomes	
ATP6a1 (ATPase, H+ Transporting, Lysosomal (Vacuolar Proton Pump), Alpha Polypeptide, 70kD)	L09235
ATP6b1(ATPase, H+ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD)	M25809
ATP6d(ATPase, H+ transporting, lysosomal (vacuolar proton pump) 42kD)	X69151
ATP6e(ATPase, H+ transporting, lysosomal (vacuolar proton pump) 31kD)	X71490
ATPase, H+ transporting, lysosomal (vacuolar proton pump) 31kD	X76228
Free radical inactivation	
Superoxide Dismutase	X02317
Maintenance of cellular redox potential at levels compatible with cell survival	

Conditionally essential genes

As indicated in the Summary, some genes are conditionally essential, meaning that they are essential for cell survival or proliferation only in certain circumstances. Most commonly such circumstances are related to changes in the environment, such as changes in the concentration of specific constituents such as nutrients, administration of pharmaceuticals (drugs), or physical elements affecting the cell. In many cases the changes in the environment may be induced as part of a treatment regiment for cancer such as the administration of drugs or ionizing radiation. In the presence of such specific environmental changes or therapies, genes with are not normally essential for cell survival or proliferation become essential and, consequently, targets for therapy under the present invention. Therapy with inhibitors of conditionally essential genes involves administration of the inhibitor together with a chemical or physical elements that causes the target gene to be essential for cell survival or proliferation. The use of allele specific inhibitors in the current invention allows specific killing of cancer cells with such chemical or physical agent since the gene function that is essential for the survival of cells (in the presence of the chemical or physical agent) is inhibited in the cancer cell but not in the normal cell.

This strategy begins with the identification of heterozygous alleles of genes coding for proteins that are conditionally essential for cell viability or growth due to change in the chemical or physical environment. In one aspect of this invention, the gene targets of this application are responsible for mediating cell response to changes in the environment. Such environmental alterations include, for example, changes in the concentration of naturally occurring constituents such as amino acids, sugars, lipids and inorganic and organic ions, as well as larger molecules such as hormones or antibodies, or changes in the partial pressure of oxygen or other gasses. The absence of a specific constituent in the environment makes the genes that are involved in synthesizing that nutrient within the cell essential,

whereas if the constituent were present in the environment in sufficient quantities, such genes would not be essential. Alternatively, high concentrations of a specific constituent in the environment may make genes that are responsible for eliminating or detoxifying that constituent within the cell essential, wheras, if the constituent were absent or present in normal concentrations, such genes would not be essential. Changes thus may involve either an increase or a decrease in specific constituents of the environments including nutrients, inorganic, or organic materials.

In another aspect of this invention, the gene targets of this application are responsible for maintaining cell survival or proliferation in the presence of a drug or biological material. For example, a drug that inhibits one pathway for maintaining the level of a cellular constituent within levels required for cell survival or proliferation may make alternative pathways essential. In a specific embodiment, the inhibition of a synthetic pathway for a cellular constituent may make alternative synthetic pathways essential for cell survival or proliferation. Alternatively, a drug that is toxic to the cell will make genes that are involved in the elimination, degradation, or excretion of the drug from the cell essential for continued survival or proliferation. It will be evident to those skilled in the art that anything which inhibits the ability of a cell to survive in the presence of a specific drug that is designed to be cytostatic or cytotoxic, will sensitize that cell to the effects of the drug. A "chemosensitizing" agent is one that inhibits a function in the cell that is conditionally essential due to the administration of a chemotherapeutic drug.

In another aspect of this invention, the gene targets of this application are responsible for maintaining cell survival or proliferation in response to external physical forces including, but not limited to, electromagnetic radiation of various amplitudes and wavelengths, including ionizing and nonionizing radiation and heating or cooling. In the presence of ionizing radiation, for example, genes that are

involved in DNA repair may be essential that are not essential in the absence of the external physical force. An agent that inhibits functions in the cell that are essential due to the adminitration of ionizing radition would be termed a "radiosensitizing" agent.

In each instance, treatment of cancer or noncancer proliferative diseases may be achieved by identifying genes that are conditionally essential in the presence of specific environmental, pharmacological, or physical factors, determining whether such genes are subject to loss of heterozygosity, identifying alternative alleles in these genes and developing allele specific inhibitors of alternative forms of the gene. The administration of such an inhibitor to a patient who has two alternative forms of the gene in normal cells but only one in the cancer cell due to LOH, together with the environmental, pharmacological or physical factors will result in an antiproliferative effect or killing of the cancer cell.

Different environmental, pharmacological, and physical changes in the environment that result in homeostatic or compensatory responses in which genes that are not normally essential for cell survival or proliferation become essential are known in the art. These are described in the following Table 2.

Table 2

1 Changes in the concentration of constituent in the environment
- Change in nutritional environment
- Change in hormonal environment
- Change in the immunological environment
- Presence or accumulation of toxic materials
- Change in partial pressure of oxygen
- Change in partial pressure of carbon dioxide.
- Change in partial pressure of other gasses including nitrous oxide

2. Administration of pharmaceuticals including small molecules, biologicals, nucleic acids, or antibodies.

3. Physical changes
   - Electromagnetic radiation
   - Ionizing radiation including Alpha particles, Beta particles, Gamma radiation
   - Non-ionizing radiation including infrared radiation, microwave radiation, other wavelengths
   - Temperature

When LOH results in a difference in normal cell genotype vs. cancer cell genotype that affects a locus encoding a product affecting the cells' ability to survive in the presence of an environmental change, a pharmaceutical or biological agent, or a physical factor, there is an opportunity to exploit a therapeutic window between cancer cells and normal cells. Below we describe specific examples of genes that (1) affect cell responses to altered environments, (2) are located on chromosomes that undergo LOH in cancer and (3) exist in two or more variant forms. These examples have been selected to illustrate how the therapeutic strategy described in this application would work with a variety of different alterations in chemical or physical environment. Example 43 describes a gene (Dihydropyrimidine Dehydrogenase) that mediates response to an altered chemical environment (presence of the toxic chemical 5-floxuridine) by specifically transforming the chemical to an inactive metabolite. Example 39 describes a gene (Methylguanine methyltransferase) that mediates response to an altered chemical environment (presence of toxic chemicals such as nitrosourea or other alkylating agents) by removing methyl or alkyl adducts to DNA, the principal toxic lesion of these agents. Example 44 describes a set of genes (Fanconi Anemia genes A,B,C,D,E,F,G and H) which mediate response to an altered chemical environment (presence of chemicals which cause DNA crosslinking, such as diepoxybutane, mitomycin C and cisplatinum) by repairing the crosslinks. Example 48 describes a set of genes (the DNA Dependent Protein Kinase Complex, including the DNA Dependent Protein Kinase catalytic subunit (DNA-PKcs), the DNA binding component (called Ku), made up of Ku-70 and Ku-86 kDa subunits, and the Ku-86 related protein Karp-1) that mediates repair of

double stranded DNA breaks, such as occurs after x-irradiation. Example 45 describes a gene (asparagine synthase) that mediates response to an altered nutritional environment (absence of extracellular asparagine) which can be produced by an enzyme such as asparaginase, which hydrolyzes serum asparagine. Example 49 describes the Ataxia Telangiectasia gene, which is involved in response to ionizing radiation and radiomimetic chemicals. Other detailed examples include methionine synthase (Ex. 46) and methylthioadenosine phosphorylase (Ex. 47). Other examples include Poly (ADP) Ribose Polymerase (PARP), Glutathione-S-Transferase pi (GST-pi), NF-kappa B, Abl Kinase, 3-alkaylguanine alkyltransferase, N-methylpurine DNA glycosylase (hydrolyzes the deoxyribose N-glycosidic bond to excise 3-methyladenine and 7-methylguanine from alkylating agent-damaged DNA polymers), OGG-1, MDR-1.

The table below presents exemplary categories and exemplary specific genes along with the type of conditions which render the gene essential.

Table 3: Categories of Conditionally Essential Genes

Genes and proteins vital for cell survival or proliferation in the presence of an altered chemical or physical environment

I. Genes required for adaptation to changes in the chemical environment

1. Adaptation to altered concentration of a naturally occuring small molecule

A. Increased concentration of a naturally occuring small molecule

i. Increased levels of amino acids
1.Targets: amino acid degradation pathways

Increased intracellular levels of amino acids can damage cells. One cause of such increased levels is failure to properly degrade amino acids into simpler compounds. Therefore an amino acid catabolizing enzyme can be a conditionally essential gene, particularly in the presence of elevated levels of the twenty amino acids commonly used in protein synthesis. Amino acid catabolic pathways are well described in textbooks and in the scientific literature.

ii. Increased levels of sugars or starches

2. Targets: mono, di and polysaccharide metabolic pathways

Galactose-1-phosphate uridyltransferase
Galactose kinase
UDPgalactose-4-epimerase

Increased intracellular levels of sugars or starches can damage cells. One cause of increased levels is failure to properly degrade starches into simple compounds, as exemplified by diseases of impaired polysaccharide metabolism. Therefore a polysaccharide catabolizing enzyme can be a conditionally essential gene, specifically in the presence of elevated levels of particular polysaccharides. A second mechanism of damage arises in the context of impaired sugar metabolism. Thus enzymes that degrade sugars or starches to simpler compounds may be conditionally essential for cell health and consequently cell proliferation. An example is the enzymes of the Leloir pathway of galactose metabolism. Mutant copies of these proteins make cells conditionally sensitive to elevated concentrations of galactose. Thus enzymes that degrade sugars or starches to simpler compounds may be conditionally essential for cell proliferation.

iii. Increased levels of vitamins

B. Decreased concentration of a naturally occuring small molecule

i. Decreased levels of amino acids

1.Targets: amino acid transporters

Decreased intracellular levels of amino acids can impair protein synthesis and thereby slow or arrest cell division. One cause of such decreased levels is impairment of cellular uptake of amino acids, particularly amino acids that the cell is not actively synthesizing, whether essential (e.g. methionine) or nonessential (e.g. asparagine; see examples). Cells have a variety of mechanisms for amino acid uptake, including membrane anchored transporters. In the presence of decreased extracellular levels of amino acids the protein and other constituents of these transporters become conditionally more essential.

2. Targets: amino acid biosynthetic machinery

a. Essential amino acids

Methionine Synthase, essential for responding to decreased extracellular methionine. (GenBank U73338)

b. Non-essential amino acid biosynthesis

Asparagine Synthase, essential for responding to decreased extracellular asparagine. (GenBank M27396)

Glutamine Synthetase, essential for responding to decreased extracellular glutamine. (GenBank Y00387)

Decreased intracellular levels of amino acids can impair protein synthesis and thereby slow or arrest cell division. One cause of such decreased levels is impairment of amino acid biosynthesis, particularly amino acids that the cell is not actively synthesizing, whether essential (e.g. methionine) or nonessential (e.g. asparagine; see examples). Cells have a variety of well described biochemical pathways for biosynthesis of the 20 amino acids commonly used in proteins. These biosynthetic enzymes can be conditionally essential in the absence of adequate intracellular levels of amino acids. Specific examples of such conditionally essential genes are described in the Examples. However, other enzymes which catalyze reactions important for maintaining levels of amino acids adequate for protein synthesis in the presence of decreased extracellular concentrations are also useful.

3. Targets: transaminases

In the presence of decreased extracellular levels of amino acids cells must increase intracellular mechanisms for amino acid biosynthesis. One such mechanism is transfer of amino groups from nonessential to essential amino acids to compensate for insufficient quantities of essential amino acids. These reactions are catalyzed by transamin-ases, which therefore can become conditionally essential in environments characterized by decreased levels of extracellular amino acids.

ii. Decreased levels of sugars

1. Targets: sugar transporters

2. Targets: sugar metabolism machinery

Increased intracellular levels of sugars or starches can damage cells. One cause of such increased levels is failure to properly degrade starches into simple compounds, as exemplified by diseases of impaired polysaccharide metabolism. Therefore a sugar or poly-saccharide catabolizing enzyme can be a conditionally essential gene in the presence of elevated levels of particular sugars or polysaccharides.

2. Adaptation to presence of non-naturally occuring molecules

A. Elimination of non-naturally occuring molecules

i. Elimination by export

Multidrug resistance gene/P glycoprotein (MDR1)
(GenBank AF016535)
Multidrug resistance associated proteins 1-5 (MRPs)
(GenBank L05628)

Cells have evolved specific mechanisms to export a variety of chemicals, including nonnatural chemicals such as cytotoxic drugs. MDR1 and MRP are exemplary ATP-dependent transmembrane drug-exporting pumps. Deficiency of these pumps is associated with increased sensitivity to a variety of cytotoxic drugs in vitro and in vivo. For example, mice lacking functional MRP are hypersensitive to the drug etoposide. Thus these pumps are important for cell survival in the presence of a variety of toxic drugs. Polymorphisms have been reported in MDR1 at amino acids 893 and 999. MDR also maps to a region of chromosome 7 which is frequently affected by LOH in prostate, ovarian breast and other cancers.

Multispecific organic anion transporters (MOATs)
Other drug export proteins

ii. Elimination by metabolic transformation

1. Specific metabolic transformation of drugs

a. Inactivation of bleomycin

Bleomycin hydrolase (GenBank U14426)

Bleomycin hydrolase was discovered through its abililty to detoxify the anticancer glycopeptide bleomycin. Cells lacking bleomycin hydrolase are highly susceptible to bleomycin toxicty (for example pulmonary fibrosis) thus the gene is conditionally essential for cell growth and survival in the presence of bleomycin. Bleomycin hydrolase is a member of the cysteine protease papain superfamily. The protein is expresed in all tissues surveyed. The crystal structure of the closely related yeast bleomycin hydrolase has been determined. A common A/G polymorphism has been described at nucleotide 1450 of the bleomycin hydrolase gene. It results in an isoleucine-valine variance at amino acid 443, part of the oligomerization domain of the homotetrameric enzyme. The Bleomycin hydrolase gene has been mapped to the proximal long arm of chromsome 17 (17q11.2), a site of frequent LOH in commonly occuring epithelial cancers such as breast and ovarian cancer.

b. Inactivation of pyrimidine analogs including 5-fluorouracil (5-FU) and 5-fluorouridine.

Dihydropyrimidine Dehydrogenase (DPD)
β_- ureidopropionase
β - alanine synthetase

DPD is described in the examples. The other two enzymes are responsible for the further metabolism of dihydro-5-fluorouracil, the metabolic product of DPD. In the absense of these enzymes toxic metabolites of 5-FU accumulate in cells.

c. Inactivation of of pyrimidine analogs including cytosine arabinoside and 5-azacytidine.

Cytidine deaminase

Cytidine deaminase (CDA) catalyzes hydrolytic deamination of cytidine or deoxycytidine. It can also deaminate cytotoxic cytosine nucleotide analogs such as cytosine arabinoside, rendering them nontoxic. Resistance to the cytotoxic effects of these drugs has been reported associated with increased expression of the CDA gene. Thus CDA is a conditionally essential gene in the presence of cytotoxic cytosine nucleotide analogs.

d. Inactivation of thiopurine drugs, including 6-mercaptopurine, 6-thioguanine and azathioprine.

Thiopurine methyltransferase (GenBank U12387)

e. Inactivation or transformation of other drugs including, but not limited to, purine analogs, folate analogs, topoisomerase inhibitors and tubulin acting drugs via specific enzymatic modification.

2. General metabolic transformation of drugs

a. Cytochrome P450 system.

CYP1

CYP1A1 (GenBank K03191)
CYP1A2 (GenBank M55053)

CYP2

CYP2A6 (GenBank U33317)
CYP2A7
CYP2B6
CYP2B7
CYP2C8
CYP2C9 (OMIM 601130)
CYP2C17
CYP2C18
CYP2C19 (OMIM 124020)
CYP2D6 (OMIM 124030)
CYP2E1 (OMIM 124040)
CYP2F1

CYP3

CYP3A3
CYP3A4 (GenBank D00003)
CYP3A5
CYP3A7

CYP4
CYP4B1
CYP7
CYP11
CYP17
CYP19
CYP21
CYP27

The cytochrome P450s are a large gene family whose members metabolically transform and inactivate a wide variety of drugs, including cytotoxic drugs. Wide variation in P450 protein expression has been described, including null alleles. For example cytochrome P450 2D6 may be involved in the metabolism of ~25% of all drugs. Between 5 and 10% of all caucasians are homozygous for completely inactive alleles of P450 2D6. In the presence of a toxic drug the P450 enzyme responsible for metabolizing the drug may be conditionally essential. For example, acute liver faillure has been reported in a patient treated with cyclophosphamide who was homozygous for the deficient CYP 2D6B allele. Liver failure was due to accumulations of a hepatotoxic 4-hydroxylated cyclophosphamide metabolite.

b. N-acetyltransferases

c. Glucuronyltransferases

d. Glutathione transferases

Glutathione transferase alpha (GenBank AF020919)
Glutathione transferase theta (OMIM 600436 & 600437)
Glutathione transferase mu (OMIM 138350, 138380, 138380, 138333 & 138385)
Glutathione transferase pi (GenBank X65032)

A large number of drugs are are biotransformed into electrophilic intermediary compounds which are potentially harmful to cell constituents unless rendered harmless by conjugation with glutathione. Thus proteins of the GST system are conditionally essential for cell survival.

B. Repair or prevention of damage by non-naturally occuring molecules

i. Repair or prevention of damage by molecules that react with nucleic acids

1. Molecules that add alkyl or other groups to DNA

a. Targets: genes & gene products involved in repair of alkylating agent damage

Methylguanine Methyltransferase (MGMT) (GenBank M29971)
3-alkylguanine alkyltransferase
3-methyladenine DNA glycosylase (GenBank M74905)

MGMT is described in the examples. hOGG1 is a DNA glycosylase with associated lyase activity that excises this adduct and introduces a strand break. Cells lacking this protein are deficient in repair of oxidative damage and have high mutation rates. In conditions of high oxidative damage, including cellular aerobic metabolism, ionizing radiation and some chemotherapy drugs the hOGG1 gene would be conditionally essential for DNA repair. The human OGG1 gene maps to chromosome 3p25, a region of high frequency LOH in lung, kidney, head and neck and other cancers. Homozygous mutant mouse cells lacking 3-methyladenine DNA glycosylase have increased sensitivity to alkylation induced chromosome damage and cell killing.

2. Molecules that induce single or double stranded DNA breaks (also relevant to survival in the presence of ionizing radiation; see below)

a. Targets: genes & gene products involved in repair of double stranded DNA breaks

DNA Dependent Protein Kinase (DNA-PK) and subunits
Catalytic subunit of DNA-PK (GenBank U47077)
DNA binding subunit of DNA-PK (Ku subunit)
Ku-70 subunit (GenBank J04611)

Ku-86 subunit (OMIM 194364/GenBank AF039597)
KARP-1
Poly (ADP-ribose) polymerase (PARP) (GenBank M32721)

b. Targets: genes & gene products that repair DNA cross-links induced by molecules such as Mitomycin C or diepoxybutane

Fanconi Anemia genes
Fanconi Anemia A gene (GenBank X99226)
Fanconi Anemia B gene
Fanconi Anemia C gene (GenBank X66894)
Fanconi Anemia D gene
Fanconi Anemia E gene
Fanconi Anemia F gene
Fanconi Anemia G gene
Fanconi Anemia H gene

4. Targets: genes & gene products required for repair of DNA damage caused by drugs such as, for example, 4-nitroquinoline -1-oxide, bromobenz(a)anthracene, benz(a)anthracene epoxide, 1-nitorpyridine-1-oxide, acetylaminofluorine and aromatic amides, benz(a)pyrene.

a. Nucleotide excision repair system
ERCC-1 (GenBank M13194)
ERCC2/XPD (GenBank X52222)
ERCC3/XPB (GenBank M31899)
ERCC4 (OMIM 133520)
ERCC5 (GenBank L20046)
ERCC6 (GenBank L04791)

b. Other DNA repair genes
XPA (GenBank D14533)
XPC (GenBank D21090)
XPE (GenBank U18300)
HHR23A (GenBank U21235)
HHR23B (GenBank D21090)
Uracil glycosylase (GenBank X52486)
3-methyladenine DNA glycosylas (GenBank M74905)

ii. Repair of damage by chemicals that interact with proteins

iii. Repair of damage by chemicals that interact with membranes

1. Free radical damage

iv. Adaptation to molecules that alter the cellular redox state (such as pyrrolidinedithiocarbamate)

3. Adaptation to change in nutritional environment

A. Decreased levels of nutrients.

B. Increased levels of nutrients.

4. Change in hormonal environment

A. Decreased levels of hormones.

B. Increased levels of hormones.

5. Change in the immunological environment

A. Introduction of new immune molecules (antibodies or antibody fragments)

B. Introduction of immune regulatory molecules

Fanconi anemia C
NF-kappa B (GenBank M58603)

Cells lacking the Fanconi anemia C gene have been shown hypersensitive to interferon gamma in vitro. Cells lacking the RelA/p65 subunit of NF kappa B are essential for preventing Tumor Necrosis Factor alpha induced cell death. Other Fanconi anemia genes or other proteins of the NF-Kappa B system and its regulators, for example I kappa B, may also mediate sensitivity to immune system molecules, for example interferons, interleukins or TNF.

II. Changes in physical environment

1. Repair of damage caused by electromagnetic radiation

A. Repair of damage caused by ionizing radiation (Alpha particles, Beta particles, Gamma radiation)

i. DNA-PK constitutents (see above)

ii. Other proteins that repair DNA damage created by DNA-PK

XRCC4 (GenBank U40622)
XRCC5/Ku80 (OMIM 194364)
XRCC6
XRCC7 (GenBank L27425)

iii. Other proteins that repair or protect from DNA damage

Glutathione-S-transferase (alpha, theta, mu and pi proteins)

Transfection of an exogenous Glutathione-S-transferase pi (GST-pi) gene is partially protective of cells treated with ionizing radiation. Thus GST activity is conditionally essential for cells exposed to ionizing radiation. Similarly, any protein that is essential for the repair of radiation induced damage or for protection of cells from radiation induced damage is a conditionally essential gene. GST activity can also affect radiation sensitivity in the presence of electron affinic drugs such as the nitroimidazoles.

I-kappa B alpha (GenBank M69043)

Increased expression of exogenous I kappa B-alpha, an inhibitor of NF-kappa B, increases cell sensitivity to ionizing radiation. Thus is conditionally essential for cells exposed to ionizing radiation. Other proteins of the NF kappa B pathway that affect radiosensitivity are likewise conditionally essential in the presence of ionizing radiation.

B. Non-ionizing radiation

i. infrared radiation

ii. ultra high frequency electromagnetic radiation (UHF)

Glutathione S transferase system (see genes listed above)

UHF electromagnetic radiation of 434 Mhz will change resonance of the glutathione cycle resulting in thiol depletion which increases radiosensivity. UHF is therefore a radiosensitizing treatment, contingent on the status of the glutathione system.

iii. Other wavelenths of electromagnetic radiation

2. Temperature

A. Heating

1. Heat shock proteins

HSP70 (OMIM 138120)

HSP27 (GenBank X54079)

B. Cooling

2. Cold sensitive proteins

3. Change in redox environment, including change in partial pressure of gasses

A. Change in partial pressure of oxygen

i. Repair of damage from reactive oxygen species

8-oxoguanine DNA glycosylase (hOGG1) (GenBank U96710)

The major mutagenic lesion caused by exposure to reactive oxygen species is 8-oxoguanine. hOGG1 is a DNA glycosylase with associated lyase activity that excises this adduct and introduces a strand break. Cells lacking this protein are deficient in repair of oxidative damage and have high mutation rates. In conditions of high oxidative damage, including cellular aerobic metabolism, ionizing radiation and some chemotherapy drugs the hOGG1 gene would be conditionally essential for DNA repair. The human OGG1 gene maps to chromosome 3p25, a region of high frequency LOH in lung, kidney, head and neck and other cancers.

Fanconi anemia genes (see above for list of 8 FA complementation groups; FA genes also mediate sensitivity to oxygen)

B. Change in partial pressure of carbon dioxide.

C. Change in partial pressure of other gases.

In addition to being hypersensitive to ionizing radiation Ataxia-Telangiectasia cells are hypersensitive to the nitric oxide donor S-nitrosoglutathione (GSNO), as are cells from some radiosensitive individuals without ataxia. GSNO induces dose-dependent DNA strand breakage; cell killing appears to be associated with formation of nitrite as the ultimate oxidation product of nitric oxide. Any protein important for response to damage induced by a dissolved gas is a conditionally essential gene in this category.

III. Identification of variances and alternative alleles.

A target gene of this invention must occur as alternative alleles in the population; that is, the DNA sequence variance should either affect the gene sequence, RNA sequence, or protein sequence of the gene or its gene products, which would facilitate the design of inhibitors of the protein product, or be a base difference anywhere within the genomic DNA sequence, including the promoter or intron regions. Such DNA sequence variance can be exploited to design inhibitors of transcription or translation which distinguish between two allelic forms of the targeted gene. Sequence variants that do not alter protein sequence can be targeted, for example, with antisense oligonucleotides or ribozymes.

The most elementary genetic variant, which is common in mammalian genomes, is the single nucleotide substitution. It has been estimated that the comparison of haploid genomes will reveal this type of variant every 300 to 500 nucleotides (Cooper, *et al.*, *Human Genetics*, 69:201:205 (1985)).

Sequence variances are identified by testing DNA from multiple individuals from

the population(s) to determine whether the DNA sequence for the target gene differs in different individuals. Many different methods for identifying gene sequence variances are known in the art, several of which are described in detail in the Examples noted below. These include, but are not limited to: (1) sequencing using methods such as Sanger sequencing which is commonly performed using automated methods (Example 37); (2) Single Strand Conformation Polymorphism (Example 28); (3) DGGE (Example 36); (4) Computational methods (Example 30); (5) Chemical cleavage, (6) HPLC; (7) Enzymatic Mutation Detection, (Example 29); (8) Hybridization; (9) Hybridization arrays; and (10) Mass spectroscopy.

Often combinations of these methods are used. For example, methods such as SSCP, DGGE, or HPLC are useful in identifying whether amplified gene segments from two individuals are identical or contain a variance. These methods do not identify the location of the variant site within the linear sequence of the amplified gene segment, nor do these methods identify the specific nature of the variance, namely the alternative bases within the variant site. Methods such as Enzymatic Mutation Detection determines where the variant site is located within the sequence, but not the specific variance. Methods such as mass spectroscopy identify the specific variance, but not it location within the segment. Methods such as sequencing, computational analysis, and hybridization arrays can determine the location of the variance and specific sequence of the variance within the segment. In addition, methods such as SSCP, DGGE, EMD, and chemical cleavage are useful for determining alleles containing more than one variant site, if such sites occur within a single amplified gene segment. For the purpose of this invention, methods have been used to identify novel variant sites within genes that are essential for cell survival or proliferation. With the above methods, the presence and type of variance are preferably confirmed, such as by sequencing PCR amplification products extending through the identified variance site.

IV. Loss of Hertozygosity

Essential genes which are located in chromosomal regions which frequently undergo LOH in a tumor or other disease or condition provide advantageous targets, as the LOH of the chromosomal region indicates that the particular gene will also undergo LOH at similar high frequency. Also, essential genes which undergo LOH at high frequencies in a particular tumor, or in a range of tumor types provide advantageous targets, as a large number of patients will be potentially treatable due to the LOH of a particular essential gene.

Cancer cells, or more broadly cells associated with certain other proliferative conditions, are generally genetically different from normal somatic cells as a result of partial or complete chromosome loss, called loss of heterozygosity (LOH), which occurs at the earliest stages of these disorders. In cancer, as a result of such early chromosome loss, all the tumor cells in an individual exhibit the same pattern of LOH since the cancer results from clonal expansion of the progenitor cell with LOH. Losses of genes in LOH range from less than 5% of a chromosome, to loss of a chromosome arm, to loss of an entire chromosome. Generally only one chromosome copy is lost, making cancer cells partially hemizygous - *i.e.*, they have only one allele of many genes. As a result of such allele loss, only the single remaining allele will be available to be expressed. Such loss of heterozygosity and other losses of genetic material in cancers is described in a variety of references, for example in Mitelman, F., Catalog of Chromosome Aberrations in Cancer, New York: Liss (1988); and Seizinger, *et al.*, "Report of the committee on chromosome and gene loss in neoplasia," *Cytogenet. Cell Genetics*, 58:1080-1096 (1991). A review of many published studies of LOH in cancer cells is provided in Lasko, Cavenee, and Nordenskjold, "Loss of Constitutional Heterozygosity in Human Cancer," *Ann. Rev. Genetics*, 25:281-314 (1991).

There is considered to be a causal relationship between LOH and the origin of

cancer or other proliferative disorders. Loss of heterozygosity commonly involves chromosomes and chromosome segment that contain at least one tumor suppressor gene in addition to many other genes that may not have any function associated with cancer but are coincidentally located in the same region of the chromosome, measured in physical distance or genetic distance, as the tumor suppressor gene. Tumor suppressor genes generally regulate cell proliferation or are involved in initiating programmed cell death when threshold level of damage occurs to the cell. The loss of tumor suppressor gene function is believed to confer a growth advantage to cells undergoing LOH, because it allows them to evade these negative growth regulatory events. It is the loss of tumor suppressor genes, and the proliferative advantage associated with loss of tumor suppressor functions, that drives allele loss or loss of heterozygosity. Loss of tumor suppressor gene function requires inactivation of both gene copies. Inactivation is usually due to the presence of mutations on one gene copy and partial or complete loss of the chromosome, or chromosome region, containing the other gene copy. (Lasko et al., 1991, *Annu. Rev. Genet.* 25:281-314)

Several tumor suppressor genes have been cloned. They include, for example, TP53 on chromosome arm 17p, BRCA1 on 17q, RB and BRCA2 on 13q, APC on 5q, DCC on 18q, VHL on 3p, and $p16^{INK4}$/MTS1 on 9p. Many other, as yet uncloned, tumor suppressor genes are believed to exist based on LOH data; research groups are currently working to identify new tumor suppressor genes at more than a dozen genomic regions characterized by high LOH in cancer cells, including generating detailed LOH maps which provide LOH information useful for this invention due to the ability to identify essential genes which map to these regions of LOH. While there is an extensive literature considering tumor suppressor genes as potential targets for anti-cancer therapy, these genes are, in general, not candidates for antiproliferative therapy under the present invention because most tumor suppressor genes are not essential for cell proliferation or survival. To the contrary,

it is the loss of tumor suppressor genes that enables the abnormal proliferation and survival of cancer cells.

The pattern of LOH for a particular cancer or tumor or other proliferative disorder is not merely random. Often, there is a characteristic pattern for each major cancer type. Certain regions, including segments of chromosomes 3, 9, 11, 13, and 17, are frequently lost in most major cancer types. Other regions, such as on chromosomes 1, 3, 5, 6, 7, 8, 9, 11, 13, 16, 17, 18, and 22, exhibit high frequency LOH in selected cancers. It is believed that the characteristic LOH patterns of different cancers reflects the location(s) of tumor suppressor genes related to the development of the particular cancer or cancer type. Thus, essential genes located in regions which are characteristically associated with LOH for a particular cancer, or other tumor are particularly advantageous targets for inhibitors useful for treatment of that cancer or tumor because such genes will also characteristically undergo LOH at high frequency. The fact that certain cancers predictably undergo LOH in specific regions of the genome, and that LOH occurs before the clonal expansion of cancers in precancerous, abnormally proliferating tissue is potentially useful for preventing cancer with allele specific inhibitors of essential genes.

The treatment method described herein is applicable to proliferative disorders in which clonal proliferation occurs and in which the proliferating cells commonly undergo LOH. Another example of a disorder which has been characterized as a proliferative disorder is inflammatory pannus in arthritic joints. The demonstration of LOH associated with such a disorder will indicate that the allele specific treatment would be appropriate for the disorder. For the application of the general allele specific inhibition strategy to such conditions (*e.g.*, selection of target gene and variance, identification of inhibitors, selection of composition and administration method appropriate for the condition and the inhibitor), the cells associated with the condition correspond with the tumor, *e.g.*, cancer cells, for the

methods described in the Summary above.

LOH has been described for such polyclonal or oligoclonal disease conditions, in particular for atherosclerosis (arteriosclerosis), for example in Hatzistamou et al., 1996, *Biochem. Biophys. Res. Comm.* 225:186-190. Using a limited set of markers located on 18 chromosomal arms (one marker per arm), it was found that 23% of atherosclerotic plaques exhibited LOH for at least one marker. This does not necessarily represent the maximum fraction of plaques which could potentially be treated with allele specific inhibitors because the study did not attempt to determine the sites of maximum LOH on each arm. LOH which is partial arm LOH not affecting the particular marker for that arm was not detected. In general, fine scale LOH studies (using closely spaced markers) have revealed more sites of high frequency LOH than coarser scale studies.

The LOH for alleles of essential genes in cancers forms the basis for the anticancer therapeutic strategy described in Housman, *supra*. When one allele of the essential gene is lost from the patient's cancer cells, the retained allele can be targeted with an allele specific inhibitor. Such an inhibitor will kill, or reduce or prevent the growth of cancer cells by abolishing the function of an essential gene. Normal cells, which retain both uninhibited and inhibited alleles, will survive or grow due to the expression of the uninhibited allele. This is clearly indicated because tumor cells having only one allelic form (after LOH) thrive, thus, normal cells will also function normally with one of two allelic forms inhibited.

A large number of high frequency LOH regions are identified in Fig. 5. If not previously known, this correlation can be determined routinely for one or more tumor types by mapping of essential genes to chromosomal regions which have been identified as having high frequency LOH, or by identifying essential genes which map to locations near markers which have been identified as undergoing high

frequency LOH in a tumor. As previously described, the LOH of a marker near an essential gene, or the bracketing of an essential gene by two markers which undergo LOH, is strongly indicative that the essential gene also undergoes LOH at a similar frequency.

TABLE 4
Loss of Heterozygosity in Human Solid Tumors By Chromosome Arm

Chromosome Region	Tumor Type	Chromosome Region	Tumor Type
1p	Breast carcinoma		
	Cutaneous melanoma (metastastic)	2	Uveal melanoma
	Medullary thyroid carcinoma: MEN2A		
	Neuroblastoma		
	Pheochromocytoma: MEN2A sporadic		
1q	Breast carcinoma		
	Gastric adenocarcinoma		

		4q	Hepatocellular carcinoma
3p	Breast carcinoma		
	Cervical carcinoma		
	Lung cancer: small carcinoma		
	non-small cell carcinoma		
	large cell carcinoma		
	squamous cell carcinoma		
	adenocarcinoma		
	Ovarian carcinoma		
	Renal cell carcinoma: familial sporadic		
	Testicular carcinoma		

5q	Colorectal carcinoma Hepatocellular carcinoma	6q	Ovarian carcinoma Primitive neuroectodermal tumor Renal cell carcinoma Testicular teratocarcinoma

9p 9q	Glioma Bladder carcinoma	10 10q	Glioblastoma multiforme Hepatocellular carcinoma Prostate cancer

11p	Adrenal adenoma Adrenocortical carcinoma Bladder carcinoma Breast carcinoma Embryonal rhabdomyosarcoma Hepatoblastoma Hepatocellular carcinoma Lung cancer: squamous cell carcinoma large cell carcinoma adenocarcinoma Ovarian carcinoma Pancreatic cancer Parathyroid tumors Pheochromocytoma Skin cancer squamous cell carcinoma basal cell carcinoma Testicular cancer Wilms tumor	12q	Gastric adenocarcinoma
11q	Insulinoma Parathyroid tumors		

13q Adrenocortical adenoma Breast carcinoma Gastric carcinoma Hepatocellular carcinoma Lung cancer: small cell carcinoma Neuroblastoma Osteosarcoma Retinoblastoma	14 Colorectal carcinoma 14q Neuroblastoma

16 Breast carcinoma 16q Breast carcinoma Hepatocellular carcinoma Primitive neuroectodermal tumor Prostate cancer	17p Adrenocortical adenoma Astrocytoma Bladder carcinoma Breast carcinoma Colorectal carcinoma Lung cancer: small cell carcinoma squamous cell carcinoma adenocarcinoma Medulloblastoma Neurofibrosarcoma: NF1 Osteosarcoma Ovarian carcinoma Primitive neuroectodermal tumor Rhabdomyosarcoma 17q Breast carcinoma Neurofibroma: NF1

18 Renal cell carcinoma 18q Breast carcinoma Colorectal carcinoma	22q Acoustic neurinoma Colorectal carcinoma Ependymoma Meningioma Neurofibroma

V. Use of variance-specific inhibitors of essential genes to treat non-malignant, proliferative conditions.

It was found in the present invention that noncancer proliferative disorders could also be targeted using such an allele specific strategy. Such conditions include, but are not limited to atherosclerotic plaques, abnormal tissue in arthritic joints, including pannus, benign tumors such as leiomyomas and meningiomas, and hyperplastic conditions such as benign prostatic hyperplasia. For most of these conditions there is evidence of a mono- or oligoclonal origin and evidence of LOH. Such evidence includes the following:

- A recent study (Hatzistamou, J., Kiaris, H., Ergazaki, M., et al. (1996) Loss of heteroxygosity and microsatellite instability in human atherosclerotic plaques. *Biochemical and Biophysical Research Communications* 225: 186-190.) demonstrated that allele loss occurs in atheromatous plaques, which have long been viewed as benign neoplastic proliferations by some investigators (Benditt, E.P. and J.M. Benditt (1973) Evidence for a monoclonal origin of human atherosclerotic plaque. *Proc. Natl. Acad. Sci. U. S. A.* 70: 1753-7). Each atheromatous plaque constitutes a separate independently arising primary lesion. Consequently, allele loss in individual atherosclerotic plaques will differ, with, for example, allele A of a hypothetical essential gene lost in some plaques and allele A' in others. An inhibitor of allele A would be expected to kill (or arrest growth of) only about half of all the plaques with allele loss at the hypothetical locus - those plaques hemizygous for A. To kill the other half of the plaques with allele loss at the target locus would require an inhibitor of A'. Simultaneous use of inhibitors of A and A' would be highly toxic to diploid normal cells. However serial use of an inhibitor directed to allele A followed by an inhibitor directed to A' (perhaps repeating treatment for several cycles, or even indefinitely) would alternately abolish essential gene function in one half of all haploid plaque cells and then the other half, leading eventually to death or sustained inhibition of proliferation of all plaque cells. Normal cells would retain

50% gene function in the presence of inhibitor (either from allele A or allele A'). This therapeutic approach is applicable to the eradication of any clonal proliferation of cells in which allele loss has rendered the cells partialy haploid.

- LOH has been described in a wide variety of premalignant conditions such as metaplasia and dysplasia of colonic epithelium, breast epithelium, lung epithelium and cervical epithelium. Most studies have focused on metaplastic or dysplastic epithelium adjacent to cancer tissue, and have shown patterns of LOH similar to those in the adjacent malignant epithelium. Prophylactic ablation of such premalignant tissues could prevent the subsequent development of cancer.
- In benign tumors such as leiomyomas and parathyroidomas, which frequently must be surgically removed, LOH has been well described. As with atherosclerotic plaques, these tumors are frequently multifocal and therefore the approach of serial inhibition of allele A followed by inhibition of allele A' would alternately abolish essential gene function in one half of all haploid tumor cells and then the other half, leading eventually to death or sustained inhibition of proliferation of all tumor cells.
- LOH has been described in endometriosis, a proliferative condition associated with pain and infertility and frequently requiring surgical removel of endometrial tissue growing outside the uterine cavity. As with atherosclerotic plaques, there is only one study published to date and the frequency of LOH is low (15-18%), however the study examined only six chromosome arms; additional studies may lead to identification of regions of higher frequency LOH
- LOH is apparently the necessary event in the development of cyts in some, and possibly all, forms of autosomal dominant polycystic kidney disease (ADPKD). (There are three forms, with ADPKD1 accounting for about

85% of cases and ADPKD2 about 15% of cases.) LOH has been demonstrated by genetic analysis of the cells lining cyst walls in kidneys of ADPKD1 patients: the cells have undergone LOH for markers flanking the ADPKD1 gene. As a result the cyst cells lack functional ADPKD1. (Patients with ADPKD inherit one defective copy of an ADPKD gene from their parents.) Only about 20% of cysts were shown to have LOH when studied with a few markers, but this likely reflects, at least to some extent, technical difficulties in obtaining pure populations of cyst cells for analysis. The extent of loss of heterozygosity in cyst cells has not been well studied; only several polymorphic markers in the vicinity of the ADPKD1 gene on chromosome 16p were tested in one study (Qian, F., Watnick, T.J., et al. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* 87:979-987, 1996.) Another study found one case of LOH on chromosome 3p, distant from the ADPKD gene. Future LOH studies may reveal more extensive LOH in ADPKD. Also, it is worth noting that, unlike malignancy where it is desirable to eradicate all disease cells, eradication of a fraction of the cysts in ADPKD would be expected to have a significant beneficial effect. This is evident from the disparate clinical presentation of ADPKD, with varying numbers of cyts being associated with varying degrees of impairment of kidney function.

- Other conditions in which LOH has been demonstrated include hamartomas in tuberous sclerosis patients, odontogenic keratocysts and pterygia (benign lesions of the corneoconjunctival limbus).
- Other conditions in which there is evidence of clonal proliferation include inflammatory pannus in arthritic joints, benign prostatic hypertrophy, and hereditary hemorrhagic telangiectasia. (Qian, F. and G.G. Germino. "Mistakes Happen": Somatic Mutation and Disease. *Am. J. Hum. Genet.* 61: 1000-1005, 1997.)

Thus, consistent with the Summary above, it was found that LOH occurs in many non-malignant neoplasias or tumors with subsequent clonal growth of cells which contain only one allelic form in individuals whose normal somatic cells are heterozygous for the particular essential gene. The essential gene can therefore be inhibited by an allele specific inhibitor, *i.e.*, a variance specific inhibitor. In some conditions, however, multiple, independently arising lesions in an individual are subjected to LOH in a disease or condition, *e.g.*, in the development of atherosclerotic plaques. For that example, in individuals heterozygous for a particular essential gene which undergoes LOH, this results in some atherosclerotic plaques in which cells have one of the allelic forms of an essential gene, and other plaques in which cells have the alternative form of the gene.

It was determined that such conditions can be treated using allele specific inhibitors despite the presence of both alleles in cells related to the condition. There are two strategies for such therapy. The first is to serially administer different inhibitors targeted to the different allelic forms of the target gene. This can be accomplished by using inhibitors which target the alternative sequence variants of one sequence variance site. Simultaneous administration of inhibitors of both allelic forms of an essential gene would inhibit the cells which have undergone LOH at that gene, but would also inhibit the normal heterozygous cells of the individual. This treatment would inhibit essential functions in normal cells as well as cancer cells and have no advantage over the administration of conventional antiproliferative drugs, many of which are inhibitors of known essential functions. In contrast, administration of the first inhibitor targets the subset of cells which have only the first allelic form of an essential gene. As described for the general strategy, this inhibitor will not significantly affect the growth or survival of the normal heterozygous somatic cells. This first administration is followed by administration of a second inhibitor; the second

inhibitor targets the cells which contain only the second allelic form of the gene, and again does not significantly affect the normal somatic cells. This process of alternating administration can be repeated as needed to achieve a desired therapeutic effect. In some cases many rounds of alternating administrations will be useful. Similarly, recurring, or even indefinitely continued alternating administrations will provide useful treatment. Likewise, these methods can incorporate the use of inhibitors targeted to specific alleles of a plurality, e.g., 2, 3, 4, or more different target genes.

In certain instances, even though the lesions in non-malignant diseases are not clonal, there may be systematic loss of one parental chromosome allowing effective therapy with only one variance-specific inhibitor. This would occur, for example, if there were an inherited or early embryonic mutation within a tumor suppressor gene on one parental chromosome, in which case any event which was associated with the elimination of the corresponding normal tumor suppressor gene on the other parental chromosome would lead to abnormal proliferation. In such cases a variance-specific inhibitor of an essential gene that was closely linked to the normal tumor suppressor gene would preferentially kill cells in the proliferating lesion.

VI. Characteristics of allele-specific inhibitors

As indicated above "allele specific inhibitors" or " allele specific anti-neoplastic agents" represent a new approach to tumor therapy because they are lethal or significantly inhibit the growth only of tumor cells. The advantages of this approach include, first, lack of toxicity to the normal cells of the patient resulting in a therapeutic index greater than that of conventional tumor, *e.g.*, cancer chemotherapy drugs, and second, it is not necessary that the inhibitors be targeted specifically to the tumor cells, as they can be administered systemically. As also described above, usually an allele specific inhibitor is specific for a single

sequence variance of an essential gene, though in some cases the inhibitor utilizes the joint effects of two or more sequence variances on a particular allele.

It is not necessary for the allele specific inhibitor to have absolute specificity. Normal cells expressing equal amounts of two allelic forms of a gene product encoded by the essential gene will often show a reduction in gene activity when they take up the inhibitors of this invention, but should remain viable due to the activity of the protein encoded by the uninhibited allele. On the other hand, tumor cells expressing only one allele due to LOH, will respond to the inhibitors of this invention which are specifically directed to the remaining allele, with a greater reduction in gene activity. Growth of tumor cells exposed to the inhibitors of this invention will be inhibited due to the suppression of either the synthesis or the biological activity of the essential gene product.

Also, while a single gene has only two allelic forms in any given individual, the gene can have more than two allelic forms in a human population. Accordingly, inhibitors can be targeted to any of the alleles in the population. A particular inhibitor will generally be targeted to a subset of the allelic forms; the members of the subset will have a particular sequence variance which provides the specific targeting. In some cases, however, the inhibitor will jointly target two, or possibly more sequence variances.

Once two or more alleles are identified for a target essential gene, inhibitors of high specificity for an allele can be designed or identified empirically. Inhibitors that can be used in the present invention will depend on whether allelic variation at a target locus affects the amino acid sequence, the mRNA sequence, or the DNA in intron and promoter regions. If there is variation at the protein level, then classes of inhibitors would include low molecular weight drugs, oligopeptides and their derivatives, and antibodies, including modified or partial

antibody fragments or derivatives. For mRNA or DNA sequence variance the main class of inhibitors are complementary oligonucleotides and their derivatives and catalytic RNA molecules such as ribozymes, including modified ribozymes. The generation of inhibitors of this invention can be accomplished by a number of methods. The preferred method for the generation of specific inhibitors of the targeted allelic gene product uses computer modeling of both the target protein and the specific inhibitor. Other methods include screening compound libraries or microorganism broths, empirical screening of libraries of peptides displayed on bacteriophage, and various immunological approaches.

Further, in the treatment of cancer patients, a therapeutic strategy includes using more than one inhibitor of this invention to inhibit more than one target. In this manner, inhibitors directed to different proteins essential to cell growth can be targeted and inhibited simultaneously. The advantage of this approach is to increase the specificity of the inhibition of proliferation of cancer cells, while at the same time maintaining a low incidence of side effects.

A. Targeted Drug Design.

Computer-based molecular modeling of target proteins encoded by the various alleles can be used to predict their three-dimensional structures using computer visualization techniques. On the basis of the differences between the three-dimensional structure of the alternate allelic forms of the proteins, determinants can be identified which distinguish the allelic forms. Novel low molecular weight inhibitors or oligopeptides can then be designed for selective binding to these determinants and consequent allele-specific inhibition. Descriptions of targeted drug design can be found, for example, in I. Kuntz, "Structure-Based Strategies for Drug Design and Discovery," *Science* 257:1078-1082 (1992) and J. Dixon, "Computer-Aided Drug Design: Getting the Best Results," *Trends in Biotechnology* 10:357-363 (1992). Specific applications of the binding of

molecules to receptors using computer modeling have been described in Piper *et al.*, "Studies Aided by Molecular Graphics of Effects of Structural Modifications on the Binding of Antifolate Inhibitors to Human Dihydrofolate Reductase," *Proc Am. Assoc. Cancer Res. Annual Meeting* 33:412 (1992); Hibert *et al.*, "Receptor 3D-Models and Drug Design," *Therapie* (Paris) 46:445-451 (1991)(serotonin receptor recognition sites). Computer programs that can be used to conduct three-dimensional molecular modeling are described in G. Klopman, "Multicase 1: A Hierarchical Computer Automated Structure Evaluation Program," Quantitative Structure-Activity Relationships, 11:176-184 (1992); Pastor *et al.*, "The Edisdar Programs Rational Drug Series Design," Quantitative Structure-Activity Relationships, 10:350-358 (1991); Bolis *et al.*, "A Machine Learning Approach to Computer-Aided Molecular Design," *J. Computer Aided Molecular Desig*, 5:617-628 (1991); and Lawrence and Davis, "CLIX: A Search Algorithm for Finding Novel Ligands Capable of Binding Proteins of Known Three-Dimensional Structure," *Proteins Structure Functional Genetics* 12:31-41 (1992).

Low molecular weight inhibitors specific for each allelic protein form can be predicted by molecular modeling and synthesized by standard organic chemistry techniques. Computer modeling can identify oligopeptides which block the activity of the product of the target gene. Techniques for producing the identified oligopeptides are well known and can proceed by organic synthesis of oligopeptides or by genetic engineering techniques. R. Silverman, The Organic Chemistry of Drug Design and Drug Action, Academic Press (1992).

The inhibitors of this invention can be identified by selecting those compounds that selectively inhibit the growth of cells expressing one allelic form of a gene, but do not inhibit the activity of the A allelic form.

B. Small Molecule Inhibitors

Low molecular weight inhibitors can be identified and generated by at least one of the following methods; (1) screening of small organic molecules present in microorganism fermentation broth for allele-specific activity; or (2) screening of compound libraries. Once a compound is identified which exhibits allele specific activity, derivatives of that compound can be obtained or produced in order to obtain compounds having superior properties, such as greater activity, greater specificity , or better administration related properties (*e.g.*, solubility, toxicity, and others).

A small molecule for allele specific targeting, *i.e.*, variance specific targeting, to a polypeptide or protein target will generally have the following characteristics:

- Differential binding affinity for protein domains altered by the amino acid variance *or* uniform binding to the protein with differential effects due to subsequent interactions with variant residues.
- Inhibition of protein function following differential binding. Several mechanisms of inhibition are possible including:
  - competitive inhibition of active sites or critical allosteric sites,
  - allosteric inhibition of protein function,
  - altering compartmentalization or stability, and
  - inhibition of quaternary associations.
- Favorable pharmaceutical properties, such as safety, stability, and kinetics.

In view of the art relating to identification of compounds that interact with particular features of a polypeptide or protein or protein complex, There are clear precedents for developing drugs, *i.e.*, inhibitors, that are variance-specific including drugs that are allosteric inhibitors of protein functions. Several lines of experimental evidence demonstrate that small molecule variance specific

inhibitors can be designed and constructed for particular targets. Specifically:

- Several essential gene targets have been identified that contain variances within domains comprising the active site.
- It is possible to screen for ligands that recognize variant surface features. Combinatorial methods using antibodies, peptides, or nucleic acids suggest that specific ligands can be selected for large fractions of the surface of any protein.
- There are many literature reports of single amino acid substitutions, within the active site as well as elsewhere within a protein, altering ligand specificity and drug action.
- Allosteric (noncompetitive) inhibition of protein function may be induced by binding ligands to many different surfaces of a protein. Ligands can cause allosteric inhibition by disturbing secondary, tertiary or quaternary (subunit-subunit) interactions of a protein. There is ample evidence that such effects can e induced by binding to sequences outside the active site and even in regions that are uninvolved in the normal catalytic or regulatory activity of a protein.

Each of these points is discussed in more detail below.

Variances located within domains comprising the active site.

Crystal structures are available for several of the exemplary targets or for homologous proteins that can allow prediction of tertiary structure. As noted, the protein variance in Replication Protein A occurs within the domain that is involved in binding DNA. The protein variance in CARS occurs within the domain involved in tRNA binding.

The proximity of the active site to these variances may be exploited by several different strategies:

- Competitive inhibitors can exert variance-specific effects by exhibiting differential affinities for variant active sites, thereby interfering with binding of the substrate or critical allosteric effectors.
- Competitive inhibitors may bind with equal affinity for the active site but exerting different effects on the structure or function of the variant domain.
- Allosteric inhibitors can exert variance-specific effects by binding differentially to variant forms of the active domain and distorting the structure or function of the active site.

Screening for ligands that recognize variant surface features.

Combinatorial libraries of antibodies, peptides, nucleic acids, or carbohydrates have been used to demonstrate that ligands can be identified that will bind to large fractions of the surface of any protein.

A library of 6.5×10^{10} antibody-bearing phage was screened for binding to various targets and contained antibodies against all targets tested.

Selex and Aptamer technologies involve selection of random oligonucleotides that bind to specific targets. Reports indicate that ligands with high affinity and specificity can be selected for diverse targets despite the limited chemical diversity of the nucleic acid-based ligands.

These studies demonstrate the ability to identify ligands for unique surface features using several different chemistries. Similarly, small molecule protein surface interaction can be screened; two broad approaches for identifying small molecule ligands can be distinguished:

- Combinatorial approaches coupled with methods for high-throughput screening provide a similar scope of opportunities as combinatorial methods focused on nucleic acids, peptides, or carbohydrates.

- Rational design or focused combinatorial approaches based on biochemical, biophysical, and structural data about the target protein may be optimal when the crystal structure of the protein is known.. When the crystal structure of the target protein or its homologues are known it will often be possible to model the topology and surface chemistry of the target in detail. These data are useful in optimizing the binding specificity or allosteric inhibitory function of the product through a series of iterative steps once a prototype binding ligand is identified. Structural modeling of the target can be particularly useful in optimizing the variance specificity of a ligand that binds to the target sequence.

Examples of single amino acid substitutions altering sensitivity to small molecules

Many amino acid substitutions have been described in proteins that alter the specificity or function of small-molecule ligands. These substitutions are useful models for variance-specific interactions (*e.g.* interactions that are altered by the amino acid substitutions that distinguish variant forms of a protein.)

There are clear precedents for variance-specific drug effects in humans. Variance-specific interactions are observed in a wide variety of structurally and functionally heterogeneous proteins. Among these are variances in human proteins including:

- N-acetyl transferase 2 - variances affect acetylation of drugs including caffeine and arylamines;
- CYP2C19 - variances affect the hydroxylation of mephenytoin and related compounds;
- CYP2D6 - variances affect hydroxylation of debrisoquine and related compounds;
- glucose-6-phosphate dehydrogenase - variances account for sensitivity to primaquine and other drugs.

There are numerous examples of variance-specific drug effects in targets for antiviral and antimicrobial drugs. The most extensively characterized are those in HIV Reverse Transcriptase and β-lactamase. These data indicate that many different amino acid substitutions can alter drug effects. Moreover, while amino acid substitutions are classically distinguished as "conservative" or "non-conservative," it is evident from these data that many seemingly "conservative" substitutions can have significant effects. For each of the types of amino acid substitution identified within the exemplary target genes, examples of the same amino acid substitution altering the interaction of small molecule drugs on a target protein is shown in one or more of the model systems.

Sites of allosteric inhibition

Most drug development focuses on *competitive* inhibitors of protein action rather than noncompetitive, *allosteric* inhibitors. There is no *a priori* advantage to a competitive versus allosteric inhibitor except for the fact that medicinal chemistry often begins with candidate molecules derived from natural substrates or cofactors. There are, in fact, conceptual advantages to allosteric inhibitors since each protein may contain multiple allosteric sites, and allosteric inhibitors may be effective at lower concentrations (*e.g.* those equivalent to the substrate) since there is no need to compete with the substrate for binding.

Detailed crystallographic and other structural studies of a variety of enzymes show that the mechanism of allosteric inhibition commonly involves conformational changes (*e.g.* domain movements) far from the site of contact with the allosteric regulator. These data illustrate the cooperativity of protein structure, demonstrating how a small change in one region of a protein is amplified throughout the structure. Such cooperativity allows small molecules binding to various regions of a protein to have significant structural and

functional effects.

One way to assess the probability of achieving allosteric effects from a variant sequence is to examine the distribution and nature of mutations that affect drug action in several well-characterized proteins. Another is to examine the distribution of epitopes for antibodies that bind to the surface of a protein and inhibit its function. Analyses of these types show that allosteric sites are widely dispersed within proteins and may comprise the majority of the protein's surface.

For example:

HIV-1 reverse transcriptase (RT) is a heterodimer with p66 and p51 subunits. The p66 subunit is 560 amino acids, and p51 is a 440 amino acid subfragment of p66. The three dimensional structure of HIV-1 RT has been solved by x-ray crystallography. Three HIV-1 RT structures have been published, including complexes with double stranded DNA at 3.0 Å resolution and with the non-nucleoside inhibitors nevirapine (at 3.5Å) and -APA (at 2.8Å).

Two classes of HIV-1 RT inhibitors have been developed. The first class comprises nucleoside analogues including AZT, ddI and ddC. The second class comprises non-nucleoside analogues belonging to several chemical groups, including TIBO, BHAP, HEPT, -APA, dipyridodiazepinone, pyridinone, and inophyllum derivatives, all of which bind the same hydrophobic pocket in HIV RT. Many amino acid substitutions have been described that produce resistance to these drugs. Table 5 shows the location of selected mutations within HIV-1 RT that cause resistance to nucleoside analogues as well as the mechanism of inhibition postulated from physical-chemical experiments and structural data; the list is not comprehensive.

Table 4

Location and postulated mechanism of amino acid substitutions which confer resistance to nucleoside analog inhibitors. trp266X - multiple substitutions.

Potential resistance mechanism

Mutation	Location of mutation	Mutation creates resistance to drug(s)	Direct effect on dNTP binding	Indirect effects via interactions with dNTP binding site	Indirect effect by
met41leu	a4	AZT		X	
lys65arg	3- 4	ddC,ddI, 3TC			X
asp67asn	3- 4 loop	AZT			X
thr69asp	3- 4 loop	ddC			X
lys70arg	3- 4 loop	AZT			X
leu74val	4	ddI			X
val75thr		ddI,ddA			
glu89gly	5a	ddI,ddA			X
ile135thr	7- 8 loop	ddI		X	
met184val	9- 10 turn	ddI, ddC	X		X
thr215tyr	11a	AZT		X	X
thr215phe	11a	AZT		X	X
lys219gln	11b	AZT	X	X	X
trp266X	-thumb	AZT			

These data demonstrate that nucleoside analog resistance arises from mutations in multiple domains. Many of the mutations are located far from the dNTP binding sites. These changes inhibit drug function by altering the conformation of the target protein in a manner analogous to those conformational changes that may be induced by an allosteric inhibitor.

Table 5 summarizes the mutations that alter the function of non-nucleoside inhibitor drugs

Table 5

Location and postulated mechanism of amino acid substitutions which confer resistance to non-nucleoside analog inhibitors.

Mutation	Mutation location	Effect of mutation	Mutation confers resistance to:
ala98gly	5b- 6 loop	flexibility	Pyridinone L-697661, Nevirapine
leu100ile	5b- 6 loop	-branch	Pyridinone L-697661, Nevirapine, TIBO R82913
lys101glu	5b- 6 loop	charge	Pyridinone L-697661, Pyridinone L-697639,
lys103asn	5b- 6 loop	charge loss	Pyridinone L-697661, BHAP U-87201,Nevirapine TIBO R82913
val106ala	6	less bulky	Nevirapine, TIBO R82913
val108ile	6	bulkier	Pyridinone L-697661, Nevirapine
glu138lys	7- 8 loop	charge	TIBO R82913
val179asp	9	charge	Pyridinone L-697661
val179glu	9	charge	Pyridinone L-697661
tyr181cys	9	less bulky	Pyridinone L-697661, BHAP U-87201, Nevirapine, TIBO R82913
tyr188cys	10	less bulky	Nevirapine
tyr188his	10	less bulky	TIBO R82913, BHAP U-87201
gly190glu	10	charge	Nevirapine
leu228phe	12	bulkier	BHAP U-90152
glu233val	13	charge	BHAP U-87201
pro236leu	13- 14 loop	flexibility	BHAP U-87201
lys238thr	14	charge	BHAP U-87201
trp266X	-thumb		TIBO R82913

It is evident from these examples that the substitutions which inhibit drug functions are distributed across several domains. Different inhibitory mechanisms have been postulated in domains throughout the protein, based on the three-dimensional structure of the protein. Most involve conformational disruption of the protein secondary and tertiary structure.

Thyrotropin receptor Naturally occurring antibodies against the thyrotropin receptor can cause activation of thyroid function (Grave's disease) or inhibition of thyroid function (Hashimoto's disease). The sites within the thyrotropin receptor that are targeted by these natural antibodies have been mapped in detail and have been tested with monoclonal antibodies. Most of the inhibitory antibodies do not interfere with binding of thyrotropin to its receptor, and thus, are allosteric rather than competitive inhibitors. Several independent classes of inhibitory antibodies have been identified that bind to epitopes within different domains of the receptor. At least one of these epitopes is in a domain that is entirely unimportant for receptor activity and can be deleted by site-directed mutagenesis without disrupting the function of the receptor. These experiments provide an explicit precedent for achieving allosteric inhibitory effects from ligands that target widely dispersed sequences within the protein.

Thermus aquaticus DNA polymerase The inhibitory activity of 24 monoclonal antibodies to *Thermus aquaticus* DNA polymerase has been investigated. The antibodies recognized 13 non-overlapping epitopes. Antibody binding to eight epitopes was inhibitory. Inhibitory antibodies mapped to several distinct domains, including the 5' nuclease domain, the polymerase domain and the boundary region between the 5' nuclease and polymerase domains. Some antibodies recognized epitopes overlapping the DNA binding groove of the polymerase. Significantly, the inhibitory antibodies recognized epitopes constituting as much as 50% of the Taq polymerase surface, and the non-inhibitory antibodies a further ~25%.

β-*lactamase* The β-lactamases are a diverse family of enzymes which catalyze the hydrolysis of the β-lactam ring of penicillin and cephalosporin antibiotics. Interactions of these proteins with various small molecule drugs have been characterized in detail as the pharmaceutical industry has worked to develop chemically modified penicillins and cephalosporins to elude inactivation by β-lactamases. In addition, a β-lactamase inhibitor (clavulanic acid) has also been introduced into clinical use.

As each new drug has been introduced into wide use, mutant β-lactamases have emerged that are resistant to the drug. Over 190 β-lactamases have been described with differential specificity for the various penicillins and cephalosporins. Many of these differ by only a few amino acids. Many different amino acid substitutions at various sites within the protein can change the substrate specificity of the enzyme.

kat G (Isoniazid resistance) The kat G protein of *M. tuberculosis* encodes a catalase-peroxidase enzyme that is one of two mycobacterial genes frequently altered in isoniazid resistant strains (the other is inhA). There are a wide variety of amino acid substitutions in katG associated with drug resistance distributed evenly across the 740 amino acids of the protein. The mechanism by which some of these substitutions inhibit katG function can be inferred from the structure of the homologous yeast and *E. coli* enzymes and knowledge of the catalytic function of the enzyme. For example, insertion of an Ile between positions 125 and 126 affects a conserved interhelical loop near the active site residues; substitutions at amino acid 275 and 315 are likely to affect the ligand access channel; substitutions at amino acid 463 may affect a N-terminal substrate binding site. Other substitutions occur in regions that are not directly related to the functional sites of the protein.

The examples described above demonstrate that small molecules can discriminate in activity between polypeptides or proteins which have one a single amino acid

difference in sequence, *i.e.*, a single amino acid sequence variance.

The application of small molecule inhibitor identification is specifically discussed in Example 39 below in connection with the methylguanine methyltransferase gene.

C. Antibody Inhibition.

Once an essential gene is identified and is determined to exist in two or more allelic forms which encode different proteins, antibodies can be raised against both allelic forms of the protein. The techniques for using a specific protein or an oligopeptide as an antigen to elicit antibodies which specifically recognize epitopes on the peptide or protein are well known. Preferably monoclonal antibodies (MABs) are used.

In one embodiment, the DNA sequence of the desired allelic form of the target gene can be cloned by insertion into an appropriate expression vector and translated into protein in a prokaryotic or eukaryotic host cell. The protein can be recovered and used as an antigen to elicit the production of specific antibodies. In another embodiment, the DNA of the desired allelic form of the target gene is amplified by PCR technology and is subsequently translated *in vitro* into protein to be used as the antigen to elicit the production of specific antibodies. A third embodiment is to use the DNA sequence of the alternative alleles as a basis for the generation of synthetic peptides representing the amino acid sequence of the alleles for use as antigen to elicit the production of specific antibodies.

Antibodies can be generated either by standard monoclonal antibody techniques or generated through recombinant based expression systems. See generally, Abbas, Lichtman, and Pober, Cellular and Molecular Immunology, W.B. Saunders Co. (1991). The term "antibodies" is meant to include intact antibody molecules of the

IgD isotype as well as antibody fragments or derivatives, such as Fab and $F(ab')_2$, which are capable of specifically binding to antigen. The antibodies so produced will preferentially bind only the protein produced in the allelic form which was used as an antigen to create the antibody. If the targeted protein is expressed on the cell surface, the antibody or antibody derivative can be tested as a therapeutic. Antibody inhibitors are most effective when they are directed against cell surface proteins or receptors. If the essential protein produced by the targeted allele is not a cell surface protein or receptor, the development of antibody inhibitors may also require the use of a special antibody-delivery system to facilitate entry of the antibody into the tumor cells. The plasma membrane that surrounds all cells is designed to limit the entrance of most compounds. Entry is generally restricted to small, non-charged molecules (absence of charge allows them to slip through the fatty membrane) or to those factors that can penetrate the cell using existing, specialized import mechanisms. The introduction into cells of much larger molecules, such as specific antibodies, other proteins, or peptides, requires appropriate delivery systems such as are known in the art. Alternatively, the structure of the variable region of allele specific antibodies can be used as the basis for design of smaller allele specific inhibitory molecules.

D. Oligopeptides

Oligopeptides can be demonstrated to have a very high degree of specificity in their interaction with functional polypeptides such as cellular enzymes, receptors or other polypeptides essential for cell viability. Methods for screening peptide sequences which have high specificity for binding to, and functional inhibition of, a specific polypeptide target have been well described previously. Scott, J.K. and Smith G.P., "Searching for Peptide Ligands with an Epitope Library," *Science* 249:386-390 (1990). These methods include the screening of M13 libraries by "phage display" of polypeptide sequences as well as direct screening of peptides or mixtures of synthetic peptides for binding to or inhibition of the target functional polypeptide.

The oligopeptides of this invention can be synthesized chemically or through an appropriate gene expression system. Synthetic peptides can include both naturally occurring amino acids and laboratory synthesized, modified amino acids.

Also provided herein are functional derivatives of a polypeptide or protein. By "functional derivative" is meant a "chemical derivative," "fragment," "variant," "chimera," or "hybrid" of the polypeptide or protein, which terms are defined below. A functional derivative retains at least a portion of the function of the protein, for example reactivity with a specific antibody, enzymatic activity or binding activity mediated through noncatalytic domains, which permits its utility in accordance with the present invention.

A "chemical derivative" of the complex contains additional chemical moieties not normally a part of the protein. Such moieties may improve the molecule's solubility, absorption, biological half life, and the like. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in Remington's Pharmaceutical Sciences (1980). Procedures for coupling such moieties to a molecule are well known in the art. Covalent modifications of the protein or peptides are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues, as described below.

Cysteinyl residues most commonly are reacted with alpha-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, chloroacetyl phosphate, N-

alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloro-mercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect or reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing primary amine containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine alpha-amino group.

Tyrosyl residues are well-known targets of modification for introduction of spectral labels by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizol and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction carbodiimide (R'-N-C-N-R') such as 1-cyclohexyl-3-(2-morpholinyl(4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residue are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Derivatization with bifunctional agents is useful, for example, for cross-linking component peptides to each other or the complex to a water-insoluble support matrix or to other macromolecular carriers. Commonly used cross-linking agents include, for example, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[p-azidophenyl) dithiolpropioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (Creighton, T.E., Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86

(1983)), acetylation of the Nterminal amine, and, in some instances, amidation of the C-terminal carboxyl groups.

Such derivatized moieties may improve the stability, solubility, absorption, biological half life, and the like. The moieties may alternatively eliminate or attenuate any undesirable side effect of the protein complex and the like. Moieties capable of mediating such effects are disclosed, for example, in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990).

The term "fragment" is used to indicate a polypeptide derived from the amino acid sequence of the protein or polypeptide having a length less than the full-length polypeptide from which it has been derived. Such a fragment may, for example, be produced by proteolytic cleavage of the full-length protein. Preferably, the fragment is obtained recombinantly by appropriately modifying the DNA sequence encoding the proteins to delete one or more amino acids at one or more sites of the C-terminus, N-terminus, and/or within the native sequence.

Another functional derivative intended to be within the scope of the present invention is a "variant" polypeptide which either lack one or more amino acids or contain additional or substituted amino acids relative to the native polypeptide. The variant may be derived from a naturally occurring polypeptide by appropriately modifying the protein DNA coding sequence to add, remove, and/or to modify codons for one or more amino acids at one or more sites of the C-terminus, N-terminus, and/or within the native sequence.

A functional derivative of a protein or polypeptide with deleted, inserted and/or substituted amino acid residues may be prepared using standard techniques well-known to those of ordinary skill in the art. For example, the modified components of the functional derivatives may be produced using site-directed mutagenesis

techniques (as exemplified by Adelman et al., 1983, *DNA* 2:183) wherein nucleotides in the DNA coding the sequence are modified such that a modified coding sequence is modified, and thereafter expressing this recombinant DNA in a prokaryotic or eukaryotic host cell, using techniques such as those described above. Alternatively, components of functional derivatives of complexes with amino acid deletions, insertions and/or substitutions may be conveniently prepared by direct chemical synthesis, using methods well-known in the art.

E. Complementary Oligonucleotides and Ribozymes

Oligonucleotides or oligonucleotide analogs which interact with complementary sequences of cellular target DNA or RNA can be synthesized and used to inhibit or control gene expression at the levels of transcription or translation. The oligonucleotides of this invention can be either oligodeoxyribonucleotides or oligoribonucleotides, or derivatives thereof, which are complementary to the allelic forms of the targeted essential gene or they can act enzymatically, such as ribozymes. Both antisense RNA and DNA can be used in this capacity as chemotherapeutic agents for inhibiting gene transcription or translation. Trojan, J., *et al.*, "Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I RNA," *Science* 259:94-97 (1993). Inhibitory complementary oligonucleotides may be used as inhibitors for cancer therapeutics because of their high specificity and lack of toxicity.

Included in the scope of the invention are oligoribonucleotides, including antisense RNA and DNA molecules and ribozymes that function to inhibit expression of an essential gene in an allele specific manner. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation or directing RNase mediated degradation of the mRNA. With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, *e.g.*, between -10 and +10 regions of the relevant

nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific interaction of the ribozyme molecule to complementary target RNA, followed by a endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead, hairpin, and other motif ribozyme molecules that catalyze sequence specific endonucleolytic cleavage of RNA sequences encoding a gene product essential for cell survival, growth, or vitality.

Specific ribozyme cleavage sites within any potential RNA target can initially be identified by scanning the target molecule for ribozyme cleavage sites, such as sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features, such as secondary structure, that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays. *See*, for example, Draper PCT WO 93/23569. For the present invention, the target site will generally include a sequence variance site as described above.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA and DNA molecules. *See*, for example, Draper, *supra.* hereby incorporated by reference herein. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the

antisense or ribozyme RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense or ribozyme cDNA constructs that synthesize antisense or ribozymes RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or methyl phosphonate rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone. Modifications may also be made on the nucleotidic sugar or purine or pyrimidine base, such as 2'-O-alkyl (*e.g.*, 2'-O-methyl), 2'-O-allyl, 2'-amino, or 2'-halo (*e.g.*, 2'-F). A variety of other substitutions are also known in the art and may be used in the present invention. More than one type of nucleotide modification may be used in a single modified oligonucleotide.

A specific application of generating inhibitors which are either complementary oligonucleotides or inhibitory oligopeptides is described in Holzmayer, Pestov, and Roninson, "Isolation of dominant negative mutants and inhibitory antisense RNA sequences by expression selection of random DNA fragments," *Nucleic Acids Research* 20:711-717 (1992). In this study, genetic suppressor elements (GSEs) are identified by random DNA fragmentation and cloning in expression plasmids.

Preferred oligonucleotide inhibitors include oligonucleotide analogues which are resistant to degradation or hydrolysis by nucleases. These analogues include neutral, or nonionic, methylphosphonate analogues, which retain the ability to

interact strongly with complementary nucleic acids. Miller and Ts'O, *Anti-Cancer Drug Des.* 2:11-128 (1987). Further oligonucleotide analogues include those containing a sulfur atom in place of the 3'-oxygen in the phosphate backbone, and oligonucleotides having one or more nucleotides which have modified bases and/or modified sugars. Particularly useful modifications include phosphorothioate linkages and 2'-modification (*e.g.*, 2'-O-methyl, 2'-F, 2'-amino).

F. Gene Therapy

Nucleic acid molecules encoding oligonucleotide or polypeptide inhibitors will also be useful in gene therapy (reviewed in Miller, *Nature* 357:455-460, (1992). Miller indicates that advances have resulted in practical approaches to human gene therapy that have demonstrated positive initial results. An *in vivo* model of gene therapy for human severe combined immunodeficiency is described in Ferrari, et al., *Science* 251:1363-1366, (1991). The basic science of gene therapy is described in Mulligan, *Science* 260:926-931, (1993).

Some methods of delivery that may be used include:

a. complexation with lipids,

b. transduction by retroviral vectors,

c. localization to nuclear compartment utilizing nuclear targeting site found on most nuclear proteins,

d. transfection of cells *ex vivo* with subsequent reimplantation or administration of the transfected cells,

e. a DNA transporter system.

A nucleic acid sequence encoding an inhibitor may be administered utilizing an *ex vivo* approach whereby cells are removed from an animal, transduced with the nucleic acid sequence and reimplanted into the animal. The liver can be accessed by an *ex vivo*

approach by removing hepatocytes from an animal, transducing the hepatocytes *in vitro* with the nucleic acid sequence and reimplanting them into the animal (*e.g.*, as described for rabbits by Chowdhury et al, *Science* 254: 1802-1805, 1991, or in humans by Wilson, *Hum. Gene Ther.* 3: 179-222, 1992) incorporated herein by reference.

Many nonviral techniques for the delivery of a nucleic acid sequence encoding an inhibitor into a cell can be used, including direct naked DNA uptake (*e.g.*, Wolff et al., *Science* 247: 1465-1468, 1990), receptor-mediated DNA uptake, *e.g.*, using DNA coupled to asialoorosomucoid which is taken up by the asialoglycoprotein receptor in the liver (Wu and Wu, *J. Biol. Chem.* 262: 4429-4432, 1987; Wu et al., *J. Biol. Chem.* 266: 14338-14342, 1991), and liposome-mediated delivery (*e.g.*, Kaneda et al., *Expt. Cell Res.* 173: 56-69, 1987; Kaneda et al., *Science* 243: 375-378, 1989; Zhu et al., *Science* 261: 209-211, 1993). Many of these physical methods can be combined with one another and with viral techniques; enhancement of receptor-mediated DNA uptake can be effected, for example, by combining its use with adenovirus (Curiel et al., *Proc. Natl. Acad. Sci. USA* 88: 8850-8854, 1991; Cristiano et al., *Proc. Natl. Acad. Sci. USA* 90: 2122-2126, 1993).

In one preferred embodiment, an expression vector containing a sequence encoding a ribozyme or an antisense oligonucleotideis inserted into cells, the cells are grown *in vitro* and then infused in large numbers into patients.

The gene therapy may involve the use of an adenovirus containing a sequence encoding a ribozyme or an antisense oligonucleotide targeted to a tumor.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adenovirus, adeno-associated virus, herpes viruses, several RNA viruses, or bovine papilloma virus, may be used for delivery of nucleotide sequences into the targeted

cell population (*e.g.*, tumor cells). Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing coding sequences. *See*, for example, the techniques described in Maniatis et. al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. (1989), and in Ausubel et. al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, recombinant nucleic acid molecules encoding protein sequences can be used as naked DNA or in reconstituted system *e.g.*, liposomes or other lipid systems for delivery to target cells (*See e.g.*, Felgner et. al., *Nature* 337:387-8, 1989). Several other methods for the direct transfer of plasmid DNA into cells exist for use in human gene therapy and involve targeting the DNA to receptors on cells by complexing the plasmid DNA to proteins. *See*, Miller, supra.

In its simplest form, gene transfer can be performed by simply injecting minute amounts of DNA (*e.g.*, a plasmid vector encoding an inhibitor) into the nucleus of a cell, through a process of microinjection. Capecchi MR, *Cell* 22:479-88 (1980). The DNA can be part of a formulation which protects the DNA from degradation or prolongs the bioavailability or the DNA, for example by complexing the DNA with a compound such as polyvinylpyrrolidone. Once recombinant genes are introduced into a cell, they can be recognized by the cells normal mechanisms for transcription and translation, and a gene product will be expressed. Other methods have also been used for introducing DNA into larger numbers of cells. These methods include: transfection, wherein DNA is precipitated with $CaPO_4$ and taken into cells by pinocytosis (Chen C. and Okayama H, *Mol. Cell Biol.* 7:2745-52 (1987)); electroporation, wherein cells are exposed to large voltage pulses to introduce holes into the membrane (Chu G. et al., *Nucleic Acids Res.*, 15:1311-26 (1987)); lipofection/liposome fusion, wherein DNA is packaged into lipophilic vesicles which fuse with a target cell (Felgner PL., et al., *Proc. Natl. Acad. Sci. USA*, 84:7413-7 (1987)); and particle bombardment using DNA bound to small

projectiles (Yang NS. et al., *Proc. Natl. Acad. Sci.* 87:9568-72 (1990)). Another method for introducing DNA into cells is to couple the DNA to chemically modified proteins.

It has also been shown that adenovirus proteins are capable of destabilizing endosomes and enhancing the uptake of DNA into cells. The admixture of adenovirus to solutions containing DNA complexes, or the binding of DNA to polylysine covalently attached to adenovirus using protein crosslinking agents substantially improves the uptake and expression of the recombinant gene. Curiel DT et al., *Am. J. Respir. Cell. Mol. Biol.*, 6:247-52 (1992).

As used herein "gene transfer" means the process of introducing a foreign nucleic acid molecule into a cell. Gene transfer is commonly performed to enable the expression of a particular product encoded by the gene. The product may include a protein, polypeptide, anti-sense DNA or RNA, or enzymatically active RNA. Gene transfer can be performed in cultured cells or by direct administration into animals. Generally gene transfer involves the process of nucleic acid contact with a target cell by non-specific or receptor mediated interactions, uptake of nucleic acid into the cell through the membrane or by endocytosis, and release of nucleic acid into the cytoplasm from the plasma membrane or endosome. Expression may require, in addition, movement of the nucleic acid into the nucleus of the cell and binding to appropriate nuclear factors for transcription.

As used herein "gene therapy" is a form of gene transfer and is included within the definition of gene transfer as used herein and specifically refers to gene transfer to express a therapeutic product from a cell *in vivo* or *in vitro*. Gene transfer can be performed *ex vivo* on cells which are then transplanted into a patient, or can be performed by direct administration of the nucleic acid or nucleic acid-protein complex into the patient.

In another preferred embodiment, a vector having nucleic acid sequences encoding an allele specific inhibitor is provided in which the nucleic acid sequence is expressed only in specific tissue. Examples or methods of achieving tissue-specific gene expression are described in International Publication No. WO 93/09236, published May 13, 1993.

VII. Utility of allele-specific inhibitors of essential genes

A. Conditions susceptible to therapy.

The fraction of all cancers could be treated with allele specific inhibitors directed against allele specific essential gene targets is a function of the frequency of the target allele and the frequency of LOH. The ideal target would be deleted in 100% of all major cancers and would exist in two allelic forms, each with an allele frequency of 0.5 so that half the population would be heterozygous. An inhibitor of one allele of such an ideal target would be a useful agent for 25% of all cancer patients. An inhibitor of the other allele of the same ideal target would be therapeutic for an additional 25% of all patients, making 50% of all patients treatable. The ideal target has so far not been identified, but we have identified many essential gene sequence variance targets which are deleted in 30-70% of several major cancers, and which are heterozygous in 25-50% of North Americans. Allele specific inhibitors of both alleles of such targets would be expected to address $0.4 \times 0.5 = 0.2$ or 20% of the relevant cancer population. The relevant cancer population often includes breast, colon and lung cancer, which sum to ~500,000 new cases per year in the United States. Thus a total available market of 100,000 patients is not unusual, and many targets would be expected to address markets of at least 50,000 patients.

The targets of this invention are suitable for treatment of many different cancers, which includes cancers of different types, as well as non-malignant proliferative

disorders, as well as being suitable for use in other applications involving targeting alternative allelic forms of a gene. The classification and nomenclature for a variety of benign and malignant tumors relevant to the present invention is shown in the following table (Table 6-1 from Robbins et al., Pathologic Basis of Disease, 3rd ed. (1984), however, the invention is not limited to these cancers or classifications.

Table 6

Tissue of Origin	Benign	Malignant
I. Composed of one parenchymal cell type		*Sarcomas*
A. Tumors of mesenchymal origin		
(1) Connective tissue and derivatives		
fibrous tissue	fibroma	fibrosarcoma
myxomatous tissue	myxoma	myxocarmo
fatty tisssue	lipoma	liposarcoma
cartilage	chondroma	chondrasarcoma
bone	osteoma	osteosarcoma osteogenic sarcoma
(2) Endothelial & related tissues		
blood vessels	hemangioma capillary cavernous sclerosing	angiosarcoma
	hemangioendothelioma	endotheliosarcoma, Kaposi's sarcoma
lymph vessels	lymphoangioma	lymphangiosarcoma
synovia		synovioma (synoviosarcoma)
mesothelium		mesothelioma (mesotheliosarcoma)
brain coverings	meningioma	
glomus	glomus tumor	

?endothelial or mesenchymal cells		Ewing's tumor
(3) Blood cells & related cells		
hematopoietic cells		myelogenous leukemia monocytic leukemia
lymphoid tissue		malignant lymphomas lymphocytic leukemia plastocytoma (multiple myeloma)
monocyte-macrophage		histiocytosis X
Langerhans' cells		?histiocytic lymphoma
(4) Muscle		?Hodgkin's disease
smooth muscle	leiomyoma	leiomyosarcoma
striated muscle	rhabdomyoma	rhabdomyosarcoma
B. Tumors of epithelial origin		*Carcinomas*
stratified squamous	squamous cell papilloma	squamous cell or epidermoid carcinoma
basal cells of skin or adnexia		basal cell carcinoma
skin adnexal glands		
sweat glands	sweat gland adenoma	sweat gland carcinoma
sebaceous gland	sebaceous gland adenoma	sebaceous gland carcinoma
epithelial lining		
glands or ducts -well differentiated group	adenoma papillary adenoma cystadenoma	adenocarcinoma papillary adenocarcinoma cystadenocarcinoma
poorly differentiated group		medullary carcinoma undifferentiated carcinoma (simplex)
respiratory tract		bronckogenic carcinoma bronchial "adenoma"
neuroectoderm	nevus	melanoma (melanocarcinoma)
renal epithelium	renal tubular adenoma	renal cell carcinoma (hypernephroma)

liver cells	liver cell adenoma	hepatocellular carcinoma
bile duct	bile duct adenoma	bile duct carcinoma (cholangiocarcinoma)
urinary tract epithelium (transitional)	transitional cell papilloma	papillary carcinoma transitional cell carcinoma squamous cell carcinoma
placental eptithelium	hydatiform mole	choriocarcinoma
testicular epithelium (germ cells)		seminoma embryonal carcinoma
II. More than one neoplastic cell type---		
mixed tumors---usually derived from one germ layer salivary glands	mixed tumor of salivary gland origin (pleiomorphic adenoma)	malignant mixed tumor of salivary gland origin
renal anlage		Wilms' tumor
III. More than one neoplastic cell type derived from more than one germ layer---teratogenous		
totipotential cells in gonads or in embryonic rests	teratoma, dermoid cyst	malignant teratoma and teratocarcinoma

Allele specific therapy can be targeted to essential genes which undergo LOH in many different tumor types, including the tumors and tumor types described in the tables above, and in Figure 3.

For the treatment of patients suffering from a tumor using an allele specific inhibitor,

the preferred method of preparation or administration will generally vary depending on the type of inhibitor to be used. Thus, those skilled in the art will understand that administration methods as known in the art will also be appropriate for the inhibitors of this invention.

B. Pharmaceutical Formulations and Modes of Administration

The particular compound, antibody, antisense or ribozyme molecule that exhibits allele specific inhibitor activity can be administered to a patient either by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of a agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of one or more symptoms or a prolongation of survival in a patient.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that

includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See *e.g.* Fingl et. al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p.1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the oncogenic disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration,

penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical

compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable

coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Factors specific for the delivery of antisense and ribozyme nucleic acids are known in the art, for example as discussed in Couture et al., WO 94/02595, which is hereby incorporated herein by reference. This reference also describes the synthesis of nucleic acid molecules having a variety of 2' modified nucleotides.

The references cited herein are incorporated by reference to the same extent as if each had been individually incorporated by reference. The invention is illustrated further by the following examples, which are not to be taken as limiting in any way. The examples, individually, and together, further demonstrate that one skilled in the art would be able to practice each of the steps in developing useful pharmaceutical products as described in the invention. Generally, the development of such a product involves the following steps:

1. Select candidate target gene essential for cell survival or proliferation.
2. Determine chromosome location and LOH frequency.
3. Identify common variance in the normal population.

4. Demonstrate antiproliferative effects from inhibition of candidate gene.
5. Design variance-specific inhibitor.
6. Achieve variance-specific antiproliferative effects in cancer cells.

EXAMPLES

Example 1. Genes required for Cell Proliferation

Many genes are involved in the process of cell proliferation and are potential targets for anti-proliferative drugs in this invention. Dividing cells progress through a repeating cycle of four stages, each of which is critical to the proliferation process. During the first phase, G1, cells ready the proteins they need to replicate their DNA, which occurs during S phase. Following S phase, cells enter G2, in which they prepare to divide into two daughter cells, each of which will contain the DNA content of the original cell. The final stage of the cell cycle is M phase, in which cells undergo mitosis. During mitosis, the cell nucleus disappears and the two sets of replicated chromosomes are separated to opposite sides of the cell. The cell then divides into two cells, the nucleus reforms in each new cell, and the cycle begins again. Cell proliferation is exceedingly complex and requires the precise coordination of many processes, including DNA synthesis, chromosome condensation and separation, and cell fission. In eukaryotic cells such as yeast, many of the proteins involved in cell division are encoded by essential genes, including those contributing to the duplication of the nucleus and the functions of microtubules, spindle pole bodies the centromere and the kinetochore.

A number of proteins are essential for cell proliferation. Proteins that are critical to this process can be divided into two classes: (i) proteins that regulate cell division; (ii) proteins that form structures involved in cell division. Proteins that regulate cell division include, but are not limited to, proteins involved in the regulation of particular

steps in the division process, such as nuclear breakdown and the transition between the different stages of mitosis, as well as proteins regulating the initiation of mitosis, such as the cylins, cyclin-dependent kinases (CDKs), and the kinases and phosphatases that regulate CDKs. Cyclin B, the cyclin-dependent kinase cdc2, and the cdc25C phosphatase are examples of proteins that regulate the initiation of mitosis. Deletion of yeast homologs of these genes is lethal, verifying their critical role in regulating the entry into mitosis. (It has been established that many human genes which encode proteins involved in highly conserved cellular processes can substitute for their yeast counterparts, and vice versa. For example such conservation has been demonstrated for components of the transcriptional apparatus, as well as components of the translational apparatus.)

Proteins that form structures involved in cell division include, but are not limited to, those involved in the processes of chromosome condensation and separation. Examples are tubulin and kinesin, which participate in the separation of chromosomes, and KIAA0165 and CDC37, involved in the spindle pole. Deletion of the yeast homolog of CDC37 is lethal.

Inhibiting the ability of a cell to divide induces, by definition, a cytostatic response, often followed by cell death. Colchicine and nocodazole are examples of drugs that inhibit microtubule function *in vitro*, thereby preventing chromosome separation and leading to cell cycle arrest during mitosis. Vinblastine and vincristine, which also inhibit microtubule function and therefore cell proliferation, have been used widely in the treatment of cancer.

Examples of genes that are involved in the process of cell proliferation, and are thus essential for cell survival or proliferation are shown in the accompanying table. Each of these genes has been disrupted in Saccharomyces cerevisiae and the mutant yeast shown to be nonviable.

Table: Genes Essential for Cell Proliferation in Yeast

Gene Name	Function of Gene Product
APC1	Component of the anaphase promoting complex.
CAK1	cdk activating kinase, activates cdc28p
CBF2, CBF3B, CSE1 CBF5, CTF13, SKP1	Essential constituents of the kinetochore protein complex Cbf3 (subunits a-d), a structural component of centromeres to which microtubules attach.
CDC14	Protein tyrosine phosphatase that performs a function late in the cell cycle.
CDC15	Essential for late nuclear division
CDC16, CDC23, CDC27	Part of anaphase promoting complex, required for Clb2p degradation and metaphase-anaphase transition.
CDC28	Essential for mitosis
CDC31	Calcium binding protein of spindle pole body (SPB), involved in SPB duplication
CDC37	Required for spindle pole duplication and passage through START.
CDC5	Protein kinase required for exit from mitosis, and operation of mitotic spindle.
CKS1	Associated with cdc28p kinase
CRM1	Chromosome region maintenance protein.
CSE1	Probable kinetochore protein, interacts with cetromeric element CDEII.
CSE4	Required for chromosome segregation.
DBF4	Regulatory subunit for cdc7p protein kinase, required for G1/S transition.
DIS3	Involved in mitotic control.
DNA43	Required for S-phase initiation or completion.
DPB11	Involved in DNA replication and an S-phase checkpoint.
ESP1, KAR1	Required for regulation of spindle body pole duplication.
IPL1	Protein kinase involved in chromosome segregation.
KRR1	Essential for cell division.
MEC1	Checkpoint protein required for mitotic growth, DNA repair and recombination.
MIF2	Centromere protein required for chromosome segregation and spindle integrity

MOB1	Required for normal cell cycle progression
MPS1	Protein kinase involved in spindle body pole duplication; also mitotic checkpoint
NDC1	Required for spindle body pole duplication; nuclear envelope component
NNF1	Nuclear envelope protein required for nuclear migration during mitosis.
NRK1	Protein kinase that interacts with cdc31p
NUF2	Component of spindle body pole required for nuclear division.
RFT1	Involved in nuclear division.
SMC1, SMC2, SMC3	Coiled coil proteins involved in chromosome condensation and segregation; required for nuclear division.
SPC42, SPC97, SPC98, SPI6	Components of spindle pole body. The latter 3 interact with microtubules, gamma tubulin & stu2p, respectively.
SPK1	Protein kinase with a checkpoint function in S and G2
STU1	Required for mitotic spindle assembly.
TEM1	Involved in termination of M-phase.

It will be evident to one skilled in the art that many genes that express essential metabolic and homeostatic functions of the cell will also be essential for cell proliferation.

Example 2. Genes required to maintain inorganic ions at levels compatible with cell growth or survival.

Inorganic Ions are Essential for Cellular Life

Inorganic ions are required for virtually all cellular processes: they are important for maintenance of cell shape and osmolality; they are prosthetic groups of a wide variety of enzymes; they are required for ATP production coupled to ion diffusion; they mediate signal transduction both from intracellular and extracellular signals. Hence maintenance of inorganic ions at physiological concentrations is essential for cell

proliferation and cell survival. The importance of maintaining physiological ion concentrations is further demonstrated by the observation that deviation from normal levels leads to cytostatic or cytotoxic effects, as demonstrated by the effects of selectively poisoning ion channels or placing cells in hypotonic or hypertonic extracellular fluid.

Inorganic Ions Must be Transported Across Membranes

Maintenance of ion concentrations at optimal concentrations within cells is complicated by the presence of membranes which, because of their hydrophobic interior, form a highly impermeable barrier to most polar molecules, including inorganic ions. Important cell membranes include the plasma membrane as well as the nuclear membrane, mitochondrial membranes, the endoplasmic reticulum and Golgi apparatus, lysosomes and vesicles of various types, all of which are essential for cell proliferation or survival. Therefore maintaining the concentration of essential polar molecules, including both organic and inorganic ions, at levels compatible with cell growth or survival requires specialized mechanisms for moving such ions across the plasma membrane and the various intracellular membrane bound compartments.

Vital components of the apparatus for maintaining ion concentrations at levels essential for cell survival include regulatory molecules that sense the concentration of ions in different cellular compartments and produce signals to increase or decrease the concentration of said ions to levels compatible with cell survival; proteins that actively or passively transport ions across membranes; and proteins that modify ions so they can be transported across membranes.

Membrane transport proteins can be divided into several categories depending on whether they require energy (provided either by ATP hydrolysis or by co-transport of ions such as sodium or protons down their electrochemical gradients), produce energy

(ATP synthetases, which are usually coupled to proton diffusion) or are energy neutral. Other categories of transporters include those that transport one or more solutes (one or more of which may be ions), gated vs. non-gated - i.e. open only transiently (ligand gated and voltage gated channels) or open continuously, allowing ions to move down their concentration and electrochemical gradients. Specific types of essential membrane transporters include uniports, which simply transport one solute from one side of the membrane to the other, and cotransports, in which the transport of one solute is dependent on the simultaneous or sequential transport of a second solute in the same direction (symport) or in the opposite direction (antiport).

Other inorganic ions, such as iron, are transported bound to carrier proteins (transferrin in the case of iron). Transport of the iron carrier protein involves a complex cycle that begins with binding of iron to transferrin, binding of the iron-transferrin complex to transferrin receptor, formation of coated pits, endocytosis of the transferrin-iron complex via the coated pits, release of iron from transferrin in endosomes upon acidification to pH 5, and then recycling of the transferrin receptor-apotransferrin complex to the surface of the cell where, at neutral pH, the apotransferrin is released from transferrin receptor into the extracellular fluid to bind more iron and participate in another cycle. Thus in the case of transferrin-mediated iron transfer there are a variety of specialized proteins which must interact in a coordinated manner for transport to occur effectively.

Some of the specific inorganic ions which must be transported across the both the plasma membrane and intracellular membranes are sodium, potassium, chloride, calcium, hydrogen, magnesium, manganese, phosphate, selenium, molybdenum, iron, copper, zinc, fluorine, iodine, chromium, silicon, tin and arsenic. Specific transporters have been identified for many of these solutes including sodium, potassium, chloride, protons, copper and iron among others.

Regulation of ion concentrations at appropriate levels is often an energy-dependent process; intracellular and extracellular concentrations may differ by 10 fold or more (see Table).

Ion Concetrations Inside and Outside a Typical Mammalian Cell

Ion	Intracellular concentration (mM)	Extracellular concentration (mM)
Cations		
Na+	5-15	145
K+	140	5
Mg++	30	1-2
Ca++	1-2	2.5-5
Anions		
Cl -	4	110

Inhibitors of Ion Transporting Proteins are Cytostatic or Cytotoxic

Blocking import of essential cell nutrients, including inorganic ions, prevents cell growth and can lead to cell death. A well studied example is blockade of iron transport by inhibition of transferrin receptor. Dividing cells require iron, and transferrin receptor-mediated uptake of iron-transferrin complexes is the principal route for iron aquisition. Iron uptake requires multiple steps, including receptor binding, endocytosis via coated pits, acidification of endosomes and consequent release of iron from transferrin, followed by recycling of transferrin receptor-apotransferrin to the cell surface for another round of binding. Each step requires the coordinated function of a variety of proteins. Anti-transferrin receptor antibodies arrest cell growth by blocking iron uptake; antitumor effects have been demonstrated *in vitro* and *in vivo* with such antibodies.

Ion pumps are another class of proteins for which cytotoxic inhibitors have been

identified. All animal cells contain a Na^+, K^+ pump which operates as an antiport, actively pumping Na^+ out of the cell and K^+ in against their concentration gradients. In coupling the hydrolysis of ATP to the active transport of 3 Na^+ out and 2 K^+ into the cell the pump is electrogenic. The electrochemical gradients generated and maintained by the Na^+,K^+ pump are essential for regulation of cell volume and for the secondary, sodium-coupled active transport of a variety of organic and inorganic molecules including glucose, amino acids and Ca^{++}. Hence the sodium potassium pump plays an essential role in cellular physiology. More than one third of a typical animal cells energy requirement is expended in fueling this pump. (Alberts et al. Molecular Biology of the Cell, Garland Publishing, New York, 1983, p.291.) Ouabain is an inhibitor of the Na^+, K^+ ATPase. It binds to the catalytic alpha 1 subunit of sodium potassium ATPase and is a potent cytotoxic drug. Cells treated with ouabain swell and eventually burst as they are unable to maintain a balance of osmotic forces because they can no longer pump out Na+. See Example 11 for a more detailed description of the essential properties of the Na^+, K^+ ATPase. Amiloride is another cytotoxic drug; it blocks the sodium-proton antiporter. Thus inhibition of proteins essential for maintaining physiologial levels of inorganic ions is toxic to cells.

Ion Transporting Proteins are Evolutionarily Conserved and Essential in Other Species

Many of the proteins required to maintain inorganic ions at physiologic levels are widely conserved in eukaryotes, reflecting an ancient and vital role. A number of gene disruption experiments in non-human cells demonstrate the importance of ion transponting proteins for cell growth and survival. For example in the yeast Saccharomyces Cerevisiae the gene encoding CDC1 protein, involved in maintaining ion homeostasis, has been disrupted resulting in non-viable yeast. Another essential yeast gene is PMA1, which encodes a H+ transporting P-type ATPase of the plasma membrane; activity of the encoded protien is rate limiting for growth at low pH.

As a result of the essential functions provided by proteins required for maintenance of inorganic ions at levels required for cell growth or survival, those genes which undergo LOH in a neoplastic disorder and which have sequence variants (nucleic acid or amino acid sequences) in a population as described above, are appropriate potential targets for allele specific inhibition, and thus can be used in the methods for identifying allele specific inhibitors and in other aspects of this invention. The provision of the exemplary ion transport genes, including sodium-potassium ATPase alpha1 subunit as well as the other genes listed in the Target Genes Table, indicates that other genes within this category or related subcategories will also be appropriate potential targets. Such a gene can be identified as an essential gene by reference to the art, or by the essential gene identification methods known in the art, examples of which are referenced herein. The LOH and sequence variance characteristics can then be readily determined by the described methods, thereby demonstrating that the gene is an appropriate potential target gene for allele specific inhibition.

Example 3. Genes required to maintain organic compounds at levels compatible with cell growth or survival.

Organic Compounds are Essential for Cellular Life

Organic compounds include the amino acids, carbohydrates, lipids, nucleosides and nucleotides, ions such as bicarbonate, vitamins such as ascorbic acid, pantothenic acid, riboflavin, nicotinamide, thiamine, vitamin B6, vitamin B12, and folate, essential nutrients such as linoleic acid and a wide variety of metabolic intermediates. Organic compounds are required for virtually all vital cellular processes: they are the building blocks of all cellular macromolecules including larger organic comounds such as proteins, starches, polynucleotides and complex lipids as well as glycolipids,

glycoproteins, lipoproteins, etc.; they are constituents of all cell structural molecules including proteins and membranes; they constitute all the metabolic intermediates in such vital cell processes as glycolysis, the Krebs cycle, oxidative phosphorylation, gluconeogenesis, the urea cycle, nucleotide biosynthesis, amino acid biosynthesis, etc. Maintaining organic compounds at levels compatible with cell growth or survival constitutes a large fraction of the work of the cell. Deviation from normal levels of organic compounds will generally have cytotoxic or cytostatic effects on cells (if the appropriate homeostatic cellular machinery for maintaining organic compounds at levels compatible with cell growth or survival is not operating to bring levels back to normal), as demonstrated by the effects of preventing transport of organic ions such as essential amino acids, vitamins or ions such as bicarbonate or blocking such processes as glycolysis or amino acid biosynthesis or transport of proteins into mitochondria, or required post-translational processing of proteins, lipids or carbohydrates.

Maintaining Organic Compounds at Levels Compatible with Cell Growth or Survival Requires Membrane Transport, Biosynthesis, Energy Extraction, Energy Production, Degradation and Excretion Pathways

Maintenance of organic compounds at optimal concentrations within cells is complicated by the presence of membranes which, because of their hydrophobic interior, form a highly impermeable barrier to most polar or charged molecules or molecules over 100 Daltons, including many organic compounds. Important cell membranes include the plasma membrane as well as the nuclear membrane, mitochondrial membranes, the endoplasmic reticulum and Golgi apparatus, lysosomes and vesicles of various types, all of which are essential for cell proliferation or survival. Therefore maintaining the concentration of essential organic compounds at levels compatible with cell growth or survival requires specialized mechanisms for moving such compounds across the plasma membrane and the various intracellular membrane bound compartments.

Vital components of the apparatus for maintaining organic compounds concentrations at levels essential for cell survival include regulatory molecules that sense the concentration of ions in different cellular compartments and produce signals to increase or decrease the concentration of said compounds to levels compatible with cell survival; proteins that actively or passively transport organic compounds across membranes; and proteins that modify or bind to organic compounds so they can be transported across membranes.

Some of the specific inorganic ions which must be transported across the both the plasma membrane and intracellular membranes are sodium, potassium, chloride, calcium, hydrogen, magnesium, manganese, phosphate, selenium, molybdenum, iron, copper, zinc, fluorine, iodine, chromium, silicon, tin and arsenic. Specific transporters have been identified for many of these solutes including sodium, potassium, chloride, protons, copper and iron among others.

The number of essential membrane proteins is not known. A crude estimate can be derived by adding up the proteins which perform essential functions enumerated above. There are many presently known organic compounds which must be transported across the cell membrane, including small molecules such as essential amino acids, lipids, sugars, the vitamins pantothenic acid, folic acid, riboflavin, nicotinamide, thiamine, vitamin B_6, vitamin B_{12} and ascorbic acid as well as larger molecules such as proteins. (It is important to note that some essential functions are performed by families of transporters with overlapping tissue expression. In such cases it may be that no one protein is essential despite the fact that the protein family collectively carries out an essential cell function. Conversely, there are likely to be a number of essential membrane proteins not yet identified.)

Examples of Genes Essential to Maintain Organic Compounds at Levels Compatible with Cell Growth or Survival, From Yeast

The yeast Saccharomyces Cerevisiae is a eukaryote which shares many genes in common with humans. Approximately 70% of the essential genes in yeast have human homologs. Many human genes can be exchanged with their yeast counterparts with minimal effects on growth in yeast or human cells. The study of essential genes in yeast is much further advanced than in mammalian systems: over half of the ~6,000 genes of Saccharomyces Cerevisiae have been disrupted and the phenotype of the resulting strains tested on minimal growth media. Over 20% of disrupted yeast genes are essential, and a significant fraction of their human counterparts are likely to be essential for cell survival. Among the yeast genes disrupted are a variety of genes that encode proteins required to maintain organic compounds at levels compatible with cell growth or survival. Many of these genes are essential for cell survival. Many of the disrupted essential yeast proteins have closely related human homologs, and it is likely that the human homologs are also essential. Specific examples of yeast genes that are essential are listed below. (This is a partial list; see the web site _____.proteome.com for an up to date list.)

The yeast ACC1 gene encodes acetyl co-A carboxylase and, like the human enzyme, is the first and rate limiting step in fatty acid biosynthesis.

The yeast DYS1 gene encodes deoxyhypusine synthase which catalyzes the first step in biosynthesis of the polyamine deoxyhypusine.

The yeast FBA gene encodes fructose-bisphosphate aldolase II, the sixth step in glycolysis, while the essential yeast genes GND1, ENO2, GPM1 and PYK1 encode 6-phosphogluconate dehydrogenase, enolase 2, phosphoglycerate mutase and pyruvate kinase (the last step of glycolysis).

The yeast ERG10 gene encodes acetyl-CoA-acetyltranferase, the first step in the mevalonate/sterol pathway. The essential ERG1 gene encodes squalene

monooxygenase, an later enzyme of the sterol biosynthesis pathway. ERG7, ERG8, ERG9, ERG11, ERG20, ERG24 and ERG25 encode enzymes on the same or related pathways.

The yeast ALG1 and ALG2 genes encode mannosyltransferases requried for N-glycosylation, and the ALG7, DPM1 and NMT1 genes encode transferases for UDP-N-acetyl-glucosamine-1-P, mannose and myristate, respectively. RAM2 encodes a protein that is a subunit of both farnesyltransferases and (with BET2) geranylgeranyltransferases.

The yeast LCB1 gene encodes serine C-palmitoyltransferase which catalyzes the first step in the pathway for synthesis of the long chain base component of shingolipids, while the yeast AUR1 gene encodes a phosphoinositol transferase also essential for shingolipid synthesis.

The yeast PRO1 and PRO2 genes encode the three enzymes of proline biosynthesis. THR1 catalyzes the first step of threonine biosynthesis.

Example 4. Genes required to maintain cellular proteins at levels compatible with cell growth or survival.

Proteins carry out a host of essential enzymatic and structural functions required for cell proliferation and cell survival. Consequently, complete inhibition of protein synthesis is eventually lethal to all cells. The requirement of dividing cells (including cancer cells) for high level protein synthesis makes them more sensitive than quiescent cells to the cytostatic and cytotoxic effects of protein synthesis inhibitors. Because the basic scheme of protein synthesis remains the same in all living organisms there are many attractive schemes for screening human targets in heterologous organisms.

Polypeptide Synthesis Occurs in Several Steps and Requires Over 100 Proteins

The machinery of polypeptide synthesis includes:

Aminoacyl tRNA synthetases, which covalently couple amino acids to their cognate tRNAs. Eukaryotic cells have two sets of tRNA synthetases, one for aminoacylation of cytoplasmic tRNAs and one for aminoacylation of mitochondrial tRNAs. Both types of tRNA synthetases are encoded in the nuclear genome.

Ribosomes, which translate mRNA into protein and integrate the action of the other components of the polypeptide polymerization machinery.

Initiation factors, which mediate the steps before the first peptide bond is formed, including formation of an initiation complex consisting of a ribosome, an mRNA and the first aminoacyl tRNA. Initiation is generally the rate limiting step in polypeptide synthesis.

Elongation factors, which function in all the reactions between synthesis of the first peptide bond and addition of the last amino acid.

Termination factors, which perform the reactions required to release completed polypeptide chains from ribosomes.

Polypeptide chaperonins and other folding factors such as isomerases, which are necessary for the proper folding (and hence function) of proteins.

Polypeptide degradation machinery, including the ubiquitin system for tagging proteins for degradation and the proteasome and its constituents for cleaving proteins targeted for degradation. As cells grow and respond to changing circumstances there is a continual need to new protein synthesis. However, without some mechanism for eliminating existing unneeded or damaged proteins cells are not able to survive or proliferate.

There are approximately 20 cytoplasmic and 20 mitochondrial tRNA synthetases, approximately 80 ribosomal proteins, and over 20 protein constituents of initiation

factors, elongation factors and termination factors. The available evidence suggests that virtually all of these proteins are encoded by single copy genes. Thus at least 150 genes and their encoded proteins are potential candidates for allele specific targeting. (Conversely, the RNA constituents of the translational apparatus - transfer RNAs and ribosomal RNAs - are encoded by multicopy genes and do not constitute targets for allele specific targeting).

Inhibitors Have Been Identified for Most Steps of Peptide polymerization and processing

Well over 100 protein synthesis inhibitors with a wide variety of structures and mechanisms of action of have been characterized in both prokaryotes and eukaryotes. Specific inhibitors have been identified for each step of translation described above. See Table from Vasquez (ref. 1) for a summary of translation inhibitors.

Inhibition of aminoacyl tRNA synthetases has been accomplished by at least three different mechanisms: amino acid analogs such as borrelidin and histidinol result in arrest of cell division by competing with natural amino acids for aminoacylation by tRNA synthetases. Inhibition of prokaryotic cell growth has also been demonstrated with RNA minihelices which mimic the acceptor stems of tRNAs. The minihelices compete with authentic tRNAs for aminoacylation by cognate tRNA synthetases. A third class of synthetase inhibitor is represented by pseudomonic acid A, a species specific inhibitor of gram positive isoleucyl tRNA synthetase produced by a gram negative organism. Pseudomonic acid A does not mimic amino acids or tRNAs, but binds to isoleucyl tRNA synthetase to inhibit peptide polymerization and processing.

Peptide polymerization and processing inhibitors that act on ribosomes include agents which bind the protein components and agents which bind or cleave the RNA components of ribosomes. An example of the former is the small

molecule drug emetine, which binds to ribosomal protein S14 and inhibits peptide polymerization and processing.

Peptide polymerization and processing Inhibitors are Cytostatic or Cytotoxic Drugs

Some of the most potent cytotoxic agents known are protein synthesis inhibitors. For example, a single molecule of ricin or diphtheria toxin is sufficient to kill a cell.

The largest class of protein synthesis inhibitors act on the elongation step of translation, with many inhibitors known for both prokaryotes and eukaryotes. Among the best studied prokaryotic elongation inhibitors are molecules belonging to the major antibiotic groups: the tetracyclines, streptomycin and other aminoglycosides, lincomycin and related compounds, erythromycin and related macrolide antibiotics and puromycin. Among the better characterized eukaryotic elongation inhibitors are toxins such as ricin and diphtheria toxin.

Cancer Chemotherapy by Inhibition of Peptide polymerization and processing

The best studied chemotherapeutic agent that acts solely by inhibiting protein synthesis is the enzyme L-asparaginase, used frequently in the treatment of acute lymphoblastic leukemia and occasionally in the treatment of other cancers. The therapeutic effect of L-asparaginase treatment is hydrolysis of serum L-asparagine to L-aspartate, with a rapidly ensuing drop in serum asparagine levels. While asparagine is not an essential amino acid, leukemia cells generally do not express asparagine synthase and are therefore reliant on importation of asparagine from serum via amino acid transporters in the plasma membrane. The effect of sudden asparagine starvation on rapidly dividing leukemia cells is to induce apoptotic death. Subsequent retreatment with L-asparaginase is generally not as effective as the initial treatment because the leukemia cells which survived the initial treatment have had time to induce expression of

asparagine synthase and are no longer dependent on external asparagine.

Examples of Genes Essential to Maintain Cellular Proteins at Levels Compatible with Cell Growth or Survival, From Yeast

The yeast Saccharomyces Cerevisiae is a eukaryote which shares many genes in common with humans. Approximately 70% of the essential genes in yeast have human homologs. Many human genes can be exchanged with their yeast counterparts with minimal effects on growth in yeast or human cells. The study of essential genes in yeast is much further advanced than in mammalian systems: over half of the ~6,000 genes of Saccharomyces Cerevisiae have been disrupted and the phenotype of the resulting strains tested on minimal growth media. Over 20% of disrupted yeast genes are essential, and a significant fraction of their human counterparts are likely to be essential for cell survival. Among the yeast genes disrupted are a variety of genes that encode proteins required to maintain proteins at levels compatible with cell growth or survival. Many of these genes are essential for cell survival. Many of the disrupted essential yeast genes have closely related human homologs, and it is likely that the human homologs are also essential. Specific examples of yeast genes that are essential are listed below. All of these genes have human homologs. (This is a partial list because the Saccharomyces gene disruption project is only halfway done; see the web site http//quest7.proteome.com for an up to date list.)

GRC5, NHP2, NIP1, RPL1, RPL25, RPL27, RPL32, RPL35, RPL7, and URP2 are yeast ribosomal proteins that have been disrupted and found to be essential.

CDC33, GCD1, GCD10, GCD11, GCD2, GCD6, GCD7, PRT1, SIS1, SUI1, SUI2, SUI3, TIF11, TIF34, and TIF5 are essential translation factors, mostly translation initiation factors that initiate translation at ATG.

EFB1 and YEF3 are translation elongation factors that have been disrupted and found essential.

SUP35 and SUP45 are essential translation termination factors.

ALA1, HTS1, DED81, THS1, VAS1, WRS1 and KRS1 are essential yeast cytoplasmic tRNA synthetases.

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Example 5. Genes required to maintain cellular nucleic acids at levels compatible with cell growth or survival.

Cellular nucleic acids including deoxyribonucleic acids and ribonucleic acids are essential elements for cell survival and proliferation. Many different genes are involved in maintaining these constituents at levels required for cell growth and proliferation including genes encoding enzymes for nucleotide synthesis, nucleotide degradation and salvage, polymerization of DNA (replication), polymerization of RNA (transcription), modifications of DNA including methylation, modifications of RNA including polyadenylation and capping, and processing or DNA and RNA. Many of these genes and their gene products are targets for conventional antiproliferative drugs.

RNA and DNA precursor Biosynthesis is Essential for Cell Proliferation

Nucleotides, the building blocks for both RNA and DNA, are essential for cell survival. Eukaryotic cells have several pathways for the production of nucleotides: de novo purine and pyrimidine biosynthesis, salvage pathways and membrane transport.

Over 50 Proteins Participate in RNA and DNA precursor Biosynthesis

The principal enzyme groups involved in RNA and DNA precursor biosynthesis are the 14 enzymes of de novo purine biosynthesis, 5 enzymes of de novo pyrimidine biosynthesis (encoded in two polypeptides) and the enzymes of the nucleotide salvage pathways, which number at least 10.

Inhibitors of RNA and DNA precursor Biosynthesis are Cytostatic or Cytotoxic Drugs Useful in Cancer Chemotherapy Many of the most clinically effective antineoplastic agents block steps in RNA and DNA precursor biosynthesis. Examples include agents which block enzymes of de novo purine and pyrimidine biosynthesis or interfere with salvage pathways. For example, hydroxyurea blocks production of deoxyribonucleotides by ribonucleotide diphosphate reductase.

Purine Biosynthesis is essential for cell proliferation

Pharmacologic inhibitors of purine biosynthesis are cytotoxic. These include drugs like azaserine and 6-diazo-5-oxo-L-norleucine (DON), glutamine analogs which inhibit three steps in purine synthesis, the most important being inhibition of the enzyme formylglycinamide ribonucleotide amidotransferase. 8-azaguanine and mycophenolic acid interfere with guanylate biosynthesis. (See Komberg, A., DNA Replication, W.H. Freeman and Company, San Francisco, 1980, for a review of drugs that inhibit purine and pyrimidine biosynthesis.) There is also evidence of the essentiality of purine biosynthesis from yeast. For example, the saccharomyces cerevisiae PUR5 gene encodes inosine 5'-monophosphate dehydrogenase, which converts inosine 5'-phosphate and NAD to xanthosine 5'-phosphate and NADH, the first reaction unique

to GMP biosynthesis. Disruption of PUR5 is lethal.

Pyrimidine Biosynthesis is essential for cell proliferation

Pharmacologic inhibitors of pyrimidine biosynthesis are cytotoxic. These include drugs like phosphonacetyl-L-aspartate (PALA) which inhibits aspartate transcarbamylase, a key enzyme in de nove pyrimidine synthesis. Also, there is evidence of the essentiality of pyrimidine biosynthesis from yeast. For example, the saccharomyces cerevisiae CDC8 gene encodes thymidylate kinase, required for synthesis of dTTP. Disruption of CDC8 is lethal.

DNA synthesis and polymerization.

Cell division clearly requires DNA polymerization to replicate the chromosomes so that each daughter cell has the same genetic makeup as the parent cell. Much of the basic machinery of DNA replication is conserved in prokaryotic and eukaryotic cells (1). Disruption of genes that encode proteins of DNA replication in yeast - including Polymerases I and III (the counterparts of human polymerases a and d), and accessory factors such as Replication Protein A and Replication Factor C - is lethal in S. cerevisiae (2). Nucleotide analogs that are incorporated into DNA are cytotoxic drugs. Examples of such analogs are the antineoplastic drug 6-mercaptopurine and arabinosyl NTPs, which interfere with DNA polymerization. Since inability to replicate DNA is lethal for growing cells, mutants in DNA replication must be obtained as conditional lethals in both prokaryotes and eukaryotes.

Second strand DNA polymerization on takes place in three main steps, each requiring different protein machinery: (1) At the start of replication an initiation complex is formed at chromosome structures called origins of replication. The parental DNA strands are transiently separated, a replication fork is formed and DNA synthesis is primed. (2) The elongation phase of replication is thought to take place in two

complexes, one moving forward on the leading strand and the other moving iteratively in the opposite direction to form the lagging strand. Elongation, then, requires replicative DNA polymerases and associated factors for unwinding and transiently stabilizing single stranded DNA, proofreading the newly synthesized template and, on the lagging strand, removing RNA primers and covalently linking adjacent newly synthesized lagging strands (Okazaki fragments). (3) During the final phase of DNA synthesis replication is terminated and the newly synthesized strands are separated.

Origin recognition complexes are formed by at least 6 origin recognition complex proteins (ORC 1 through 6) along with other factors, including "licensing" proteins such as the MCM family as well as "regulating" factors.

The two principal nuclear replicative polymerases are DNA polymerase a, which is responsible for priming synthesis and for synthesis of the lagging strand, and DNA Polymerase d, which synthesizes the leading strand. Both are multisubunit proteins, which function in multiprotein assemblies that include Replication Protein A, Replication Factor C, Proliferating Cell Nuclear Antigen and other proteins.

DNA Polmerases b and e are believed to principally carry out nuclear repair synthesis, while Polymerase g is the mitochondrial replicative enzyme. These polymerases are also multiprotein complexes.

Proteins such as topoisomerases I and II and other DNA helicases are also required during replication to maintain DNA topology.

The biochemistry of replication termination is not well characterized however the proteins which carry out this final step of replication are likely to be essential.

Inhibitors Have Been Identified for Several Steps of DNA Replication

In addition to lethal disruptions of genes encoding proteins required for replication, a variety of cytotoxic inhibitors of DNA replication have been identified. They include

agents which act on production of DNA precursors as well as inhibitors of DNA polymerases.

DNA Replication Inhibitors are Cytostatic or Cytotoxic Drugs

There are several chemotherapy drugs that arrest DNA replication and poison cells by inhibiting production of deoxynucleotides, the precursors of DNA. These drugs include hydroxyurea, which inhibits ribonucleotide reductase, and 5-fluorouracil, which inhibits thymidylate synthase. Other inhibitors of replication appear to act, at least in part, by blocking DNA polymerases. These include nucleotide analogs that block DNA polymerases, such as 2',3' dideoxy NTPs and 3' deoxy ATP (cordycepin) as well as the chemotherapy drugs cytarabine (cytosine arabinoside), fludarabine phosphate and 2-chlorodeoxyadenosine. Cytarabine, after metabolism to the di- and trinucleotide phosphate forms, is incorporated into DNA and inhibits chain elongation leading to cell death, apparently by inducing apoptosis. Fludarabine, after metabolism to the triphosphate derivative, inhibits DNA polymerase, DNA primase and ribonucleotide reductase and is incorporated into DNA and RNA (3).

DNA polymerization is essential for cell proliferation

The essentiality of the function of DNA polymerization is clear, as such polymerization is needed for cell division, and therefore for tissue or tumor growth. As indicated for other categories, confirmation of the essentiality of a particular gene and the presence of a single locus, along with the determination of appropriate LOH and sequence variance heterozygosity characteristics identifies or confirms a gene in this category as an appropriate gene for potential allele specific targeting.

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Maintaining RNA at levels required for cell growth or survival

Gene transcription is necessary for the production of messenger RNAs, the precursors of all cellular proteins. Transcription is also required for the production of ribosomal RNA, essential to formation of ribosomes, and for the production of transfer RNA, required for formation of aminoacyl tRNAs, the building blocks of protein synthesis. Turning off transcription - which can be accomplished with drugs that act on DNA templates or RNA polymerase - leads to rapid arrest of cell growth and subsequent cell death. Beyond gene transcription lie a series of essential RNA processing steps, including, but not necessarily limited to, mRNA splicing, capping, polyadenylation and export to the cytoplasm. Interference with any of these steps prevents the production of mature mRNA competent for translation, and therefore has the same cytotoxic effects as blocking transcription.

Gene Transcription and RNA Processing Require Many Proteins

Transcription of eukaryotic genes is carried out by three different RNA polymerases, each of which works with a different set of accessory factors. RNA Polymerase I is responsible for transcription of ribosomal RNAs, RNA Polymerase II transcribes protein coding genes and RNA Polymerase III transcribes transfer RNAs and other small RNAs. All three polymerases are multiprotein complexes. Several protein subunits are common components of all three polymerases, but each polymerase also

has unique subunits and accessory factors, not all of which have yet been identified or characterized. Some of the key proteins identified so far are:

RNA Polymerase I subunits and accessory factors including UBF1 and SL1. (SL1 has been shown to consist of TATA binding protein and three TATA associated factors.)

RNA Polymerase III subunits and accessory factors including TFIIIA, TFIIIB and TFIIIC.

RNA Polymerase II and its accessory factors are by far the most extensively characterized and most complex system. The large multisubunit protein complex that transcribes protein coding genes has recently come to be called the RNA Pol II holoenzyme (reviewed by Berk, ref. 1). The holoenzyme consists of more than 50 proteins, among which are:

RNA polymerase, the catalytic complex at the core of the holoenzyme. It consists of 14 subunits, many of which can complement their yeast counterparts *in vivo*.

The general transcription factors. These are proteins which either make direct contact with DNA, like TATA binding protein and associated factors, or interact with other transcription factors and/or transcriptional regulators. The general transcription factors, including TFII A, TFII B, TFII D, TFII E, TFII F, TFII H and TFII I, are multimeric protein complexes with >30 protein constituents (2,3). For example, there are 8-13 proteins which associate with TATA binding protein (called TATA associated factors, or TAFs) to collectively make up TFII D. Some of these factors (e.g. TFII250) have already been proven essential for cell proliferation.

Accessory proteins such as elongation factors, termination factors, activator and mediator proteins, srb (suppressor of RNA Polymerase B; see ref. 1 and references therein) proteins, RNA methylases and a variety of other processing factors.

RNA helicases, which are required for proper folding of RNAs,

Once transcribed, genes are spliced by multiprotein assemblies termed spliceosomes (4), which are made up of pre-mRNA, small nuclear ribonucleoproteins including (snRNPs) U1, U2, U4/6 and U5 and other proteins including SF2/ASF, U2AF and SC35. Recently progress has been made in cloning cDNAs for several splicing factors, however many of the proteins which process mRNAs have not yet been well characterized. After splicing, mRNAs are polyadenylated and exported to the cytoplasm (5). Several of the proteins of polyadenylation have been purified and cloned. The export of mRNAs is less well studied but is clearly a specific process requiring protein machinery. Several essential yeast genes required for mRNA transport have been identified.

Inhibitors Have Been Identified for Several Steps of Gene Transcription

The best studied inhibitors of gene transcription are small molecules that inhibit RNA polymerase or interact with DNA to block transcription. Inhibitors of RNA polymerase include actinomycin D, which intercalates into double stranded DNA and blocks the movement of RNA polymerase and rifampicin, an antibiotic which binds the b subunit of *E. Coli* RNA polymerase and blocks initiation of transcription. The best studied specific inhibitor of eukaryotic RNA Polymerase II is the potent mushroom toxin a-amanitin, a cyclic octapeptide which binds to the polymerase with high affinity (Kd ~10-9 M). Several mutations conferring resistance to alpha-amanitin have been characterized and they all map to the RNA Polymerase II protein coding sequence.

Examples of essential yeast genes (disruption shown to be lethal) required to maintain cellular nucleic acids at levels compatible with cell growth or survival

A number of yeast genes involved in DNA (including nuclear DNA and mt DNA) and RNA (including mRNA, tRNA and rRNA) metabolism have been disrupted and shown

essential for yeast cell viability. Many of these genes are conserved in all eukaryotes. Human homologs of these yeast genes are likely to be essential for human cell growth or survival. Specific examples:

The yeast DNA2 gene encodes a DNA helicase required for DNA replication. DNA2 is essential to the function of TOP2 (topoisomerase) which is also an essential gene.

POL1, POL2, POL3 and POL12 encode DNA polymerases. The disruption of any one of these genes is lethal. Knockout of polymerase associated genes DBP2 and POB3 is also lethal. These genes are essential for the synthesis of DNA.

ORC1, ORC2, ORC3, ORC4, ORC5, ORC6, CDC7, CDC46 and CDC54 are essential in yeast. These genes encode origin recognition complex proteins responsible for the initiation of DNA synthesis. There are direct human homologs of the ORC genes.

General replication factors RFA1, RFA2, RFA3, RFC1, RFC2, RFC3, RFC4 and RFC5 are all essential yeast genes. These genes encode replication protein A and replication factor C which are essential for DNA replication and have direct human homologs.

TBF1, TEL2 and CDC13 are essential yeast genes that encode proteins that responsible for the synthesis and maintenance of telomeres.

RNR1 (Ribonucleotide Reductase 1), RNR2 (Ribonucleotide Reductase 2) CDC8 (Thymidylate Kinase) and PUR5 (Inosine-5'-monophosphate dehydrogenase) are essential yeast genes involve in the purine/pyrimidine biosynthesis pathways and in the conversion of ribonucleotides to deoxyribonucleotides.

ROX3, RPA135, RPA190, RPA43, RPB10, RPB11, RPB2, RPB3, RPB5, RPB6,

RPB7, RPB8, RPC10, RPC128, RPC19, RPC25, RPC31, RPC34, RPC40, RPC53, and RPC82 are subunits of RNA polymerases I, II and III. These genes have been disrupted and shown to be essential. RNA polymerase I, II, and II are responsible for the synthesis of rRNA, mRNA, and tRNA respectively and have human homologs.

BRR2, DBP5, DBP6, DED1, HCA4, MAK5, and ROK1 are RNA helicases that are essential for processes such as pre-mRNA splicing and ribosomal RNA splicing.

Yeast TATA binding proteins TAF145, TAF17, TAF19, TAF25, TAF40, TAF47, TAF47, TAF60, TAF61, TAF67, and TAF90 are required for mRNA transcription by the RNA Polymerase II holoenzyme.

Transcription elongation factors RPO21 and RPO31 are essential.

General transcription factors SPT15, SSL1, SSL2, SUA7, TFA1, TFA2, TFB1, TFB2, TFB4, TFC2, TFC3, TFC4, TFC5, TFG1, TFG2, TOA1, and TOA2 have been disrupted and proven to be essential. These genes encode proteins that constitute the general machinery of RNA transcription.

Specific transcription factors BBP1, BRF1, BUR6, CDC39, HSF1, KIN28, MET30, RAP1, and REB1 are essential yeast genes. These genes encode proteins that are involved in the transcription of specific genes.

CUS1, GIN10, MSL5, PRP19, PRP31, SLU7, SME1, SNP2, USS1, and YHC1 are essential genes responsible for normal RNA splicing.

ESS1, FIP1, NAB2, NAB3, NAB4, PAP1, RNA14, RNA15, and YTH1 are essential genes required for RNA modification. The encoded proteins perform functions such as cleavage and polyadenylation of 3' ends of RNAs to produce mature mRNA

molecules.

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Example 6. Genes required to maintain integrity and function of cellular and subcellular structures compatible with cell growth or survival.

In order to survive and grow cells must be able to maintain their shape and internal architecture, including the structural integrity of a wide variety of subcellular organelles including the nucleus, mitochondria, endoplasmic reticulum and Golgi vesicles and a variety of lysosomes, peroxisomes vesicles and vacuoles. These structures perform essential functions such as:

(i) Movement of proteins and other macromolecules across membranes to maintain their concentration at levels compatible with cell growth or survival. Newly synthesized proteins are transported to the endoplasmic reticulum by specialized transport apparatus which assists in protein folding and posttranslational modification. From the ER, proteins may be transported to distant cellular sites via vesicles which are comprised of specialized proteins. Some proteins synthesized in the cytoplasm must be transported into the mitochondia for proper mitochondrial function. There

also exist specialized apparatus for transport of mRNA from the nucleus.

(ii) Fusion or fission of various membrane bound cytoplasmic or nuclear organelles requires the specialized function of molecules that affect membrane properties to allow joining or separating and that provide a scaffold for moving membrane bound structures together or apart. The relationship of the ER and Golgi vesicles involves a continuous process of fission, while various classes of vacuoles or vesicles may fuse.

(iii) There must be effective coordination of the function of all cellular compartments. Coordination is accomplished by the transmission of signals from membrane to nucleus, from cytosol to mitochondria, from nucleus to cytoplasm, etc. Signals are transmitted by enzymes such as adenylate cyclases, protein kinases and protein phosphatases.

(iv) Maintenance of the integrity of cellular and subcellular structures also requires processes and structures for eliminating, transforming, sequestering or otherwise regulating levels of endogenous cellular toxins or waste substances. This may be accomplished by transfer of waste molecules to organelles such as vacuoles, lysosomes or peroxisomes, by inactivation of toxic byproducts of oxygen metabolism such as free radicals or by export of molecules that have reached excessive levels in the cell.

(v) The structure of the cytoplasm is maintained by the cytoskeleton, while different organelles in some cases are made up of specialized structural molecules. For example, the nucleus, bound by a double layered nuclear envelope, contains the nuclear matrix, consisting of over 100 unique proteins, as well as the histones and other proteins which form chromatin and the proteins which form subnuclear structures such as nucleoli, nuclear pores and the protein structures which convey mRNA out of the nucleus. (Darnell, J. et al., Molecular Cell Biology, Scientific American Books, 1990.)

The fibrous proteins of the cytoplasm are collectively referred to as the cytoskeleton. Among the important cytoskeletal proteins are microfilaments made up of actin molecules, microtubules made up of tubulin molecules, and intermediate filaments, made up of one of a variety of subunit types. The cytoskeleton is important not only

for maintenance of cell shape, strength and rigidity but also for providing a frame for movement of other structures. Microtubules, for example, are critical for chromosome movement during cell division, while actin microfilaments and intermediate filaments affect the organization and mobility of surface membrane proteins. Actins and other cytoskeletal proteins are vital for processes such as endocytosis, which is the only route of essential nutrients such as transferrin-bound iron. Cells also contain a variety of proteins essential for anchoring organelles to the cytoskeleton, or anchoring the plasma membrane to adjacent structures such as basement membranes and adjacent cells.

A variety of yeast structural proteins required to maintain integrity and function of cellular and subcellular structures have been disrupted and shown essential for cell survival. Since most structural proteins are highly conserved in eukaryotes it is likely that the human counterparts of these yeast genes are also essential. Specific examples:

The genes encoding yeast nuclear pore proteins (nucleoporin) NIC96, NSP1, NUP49, NUP57, NUP82, NUP145 and NUP159 are lethal when disrupted, as is the pore trafficking protein GSP1. NNF1 is an essential protein of the nuclear envelope required for proper nuclear morphology.

The yeast nucleolar protein NOP2, homologous to human proliferation associated nucleolar antigen p120, is essential. NOP4 encodes another essential yeast nucleolar protein.

Knockout of the yeast ACT1 gene, which encodes actin, is lethal, as is knockout of the actin related proteins ARP100, ARP2, ARP3 and ARP4. The actin binding and severing protein cofilin, encoded by the yeast COF1 gene, is also essential, as is profilin (PFY1), which can complex with actin monomers and prevent actin polymerization. PAN1 is an essential protein involved in normal regulation of the actin cytoskeleton.

The RET1, RET2, RET3, SEC1, SEC4, SEC5, SEC6, SEC7, SEC8, SEC10, SEC11, SEC14, SEC16, SEC17, SEC18, SEC20, SEC26, SEC27, SEC31, SEC61, SEC62, SEC63, SFT1, SLY1, BET1, BET3, UFE1, USO1, VTI1, TIP20, KAR2 and BOS1 genes are all essential in yeast. These genes encode proteins which are vital for the function of the endoplasmic reticulum and Golgi vesicles, including processes such as protein transport across the ER, membrane fusion and formation of vesicles.

The essential yeast histone-like protein CSE4 is required for chromosome segregation. STH1, RSC6 and RSC8 are components of the essential abundant chromatin remodeling complex, while SPT5 and SPT6 influence gene expression through effects on chromatin structure.

The essential yeast intermediate filament protein MDM1 is involved in organelle inheritance and mitochondrial morphology.

The essential yeast mitochondrial proteins MGE1 and SSC1 participate in folding of proteins during mitochondrial import. TIM17, TIM22 and TIM23 are essential mitochondrial inner membrane proteins involved in import and translocation of proteins. ATM1 is an ATP binding mitochondrial inner membrane protein.

The RAT1, MTR2 and MTR3 genes encode proteins essential for mRNA transport from the nucleus to the cytoplasm.

DBF8 is an essential yeast protein involved in protein transport.

APS2 is an essential clathrin associated protein, involved in membrane transport.

The yeast PKC1 gene encodes the essential protein kinase C, which regulates the MAP kinase cascade; CDC15 is an essential component of the MAP kinase kinase kinase

family of signaling proteins.

CYR1 is an essential adenylate cyclase which generates cAMP in response to signals including ras activation. GDI1 is an essential GDP dissociation inhibitor.

Example 8: Validation of Target Gene Essentiality

To investigate whether specific target genes are essential for cell proliferation and/or survival, a method was developed to use antisense oligonucleotides to inhibit gene expression. Phosphorothioate antisense oligonucleotides targeting polymorphic sites were transfected into human cell lines, and mRNA down-regulation was assessed by northern blotting. mRNA down-regulation was achieved for 19 of the 35 polymorphisms targeted (54.2%). Oligonucleotides targeting each polymorphic allele were (separately) transfected to assess the allele-specificity of the mRNA down-regulation. In 15 of the 19 sites accessible to oligos, the oligonucleotide targeting the allele found in the cell down-regulated mRNA to a level significantly lower than did the mismatched oligonucleotide. In 6 of these 15 cases, striking allele-specificity was observed.

The consequence of down-regulating the mRNA of an essential gene should be cell death. Allele-specific cell death was indeed observed in these experiments, both upon transfecting cells daily for three days with the phosphorothioate oligos described above (followed by a recovery period during which control-treated cells continued to divide while essential gene inhibition prevented division) or upon extended (5-10 days) daily transfections with less toxic oligonucleotide chemistries. In an experiment targeting either RNA polymerase II or the glutamyl-prolyl tRNA synthetase (EPRS), cells were transfected for five consecutive days with oligos either targeting the allele found in the cell (match) or carrying a 1 bp mismatch, targeting the other allele (mismatch). One day after the fifth transfection, cells remaining on the plate were quantitated by staining

with sulforhodamine B. The matched oligonucleotide was significantly more cytotoxic than the mismatched oligonucleotide.

Example 9: Aminoacyl tRNA Synthetases are Essential for Cell Survival

Each aminoacyl-tRNA synthetase performs an analogous role in protein synthesis, and each represents a target for the present invention.

Aminoacyl-tRNA synthetases perform a basic cell function

Aminoacyl-tRNA synthetases are present in all living cells (1). (A recent paper entitled "A minimal gene set for cellular life derived by comparison of complete bacterial genomes" [ref. 2] concludes that as few as 256 genes may be required for prokaryotic cell life; all 20 tRNA synthetases are included in this minimal gene set.) Each tRNA synthetase catalyzes ATP dependent covalent attachment of a specific amino acid to its cognate tRNA. It is the specificity of each synthetase for a single amino acid and transfer RNA that establishes the universal rules of the genetic code. The aminoacyl-tRNAs produced by tRNA synthetases constitute the precursors for protein assembly by ribosomes - thus tRNA synthetases are vital for peptide polymerization and processing. Blockade of peptide polymerization and processing at any one of multiple different steps (see above) results in arrest of cell growth and eventually cell death in a variety of organisms and cell types.

Aminoacyl-tRNA synthetases have been shown essential in all tested organisms

It has been demonstrated by mutagenesis experiments that tRNA synthetases are essential for prokaryotic, yeast and mammalian cell survival (ref. 1-5). The most relevant data concerns mammalian cells: mutagenesis of Chinese hamster ovary (CHO)

and Chinese hamster lung cells followed by "suicide" selection at 39oC for temperature sensitive (ts), conditionally lethal protein synthesis mutants has led to isolation of cell lines with mutant tRNA synthetases (reviewed in ref. 5). (The "suicide" of dividing cells is accomplished by adding thialysine or tritiated [3H] amino acids to cell growth media. Only cells that incorporate these amino acid analogs into protein die - thus cells that are protein synthesis deficient at 39oC survive the selection.) The fraction of cells surviving a single round of suicide selection ranges from one in 105 to one in 108. Biochemical and genetic characterization of surviving cells has led to identification of specific ts aminoacyl-tRNA synthetase mutants. Cell lines with mutant leucyl- or asparaginyl-tRNA synthetases have been isolated repeatedly because the genes for leu and asn tRNA synthetases are haploid in the CHO cell line used for selection, and therefore require only one mutation. Less frequently, mutant alanyl-, arginyl-, glutaminyl-, histidyl-, lysyl-, methionyl-, tryptophanyl- and valyl-tRNA synthetases have been isolated. The properties of these mutant cell lines are similar: when shifted to 39oC, the non-permissive temperature, the rate of protein synthesis drops, in some cases to almost undetectable levels. Soon thereafter the cells stop replicating DNA and within a few days cell death ensues. These experiments constitute proof of the essential role of tRNA synthetases in mammalian cells. Arrest of protein synthesis and consequent cell death can be prevented in some cases by supplementing cell media with the amino acid substrate of the defective tRNA synthetase (thereby driving the aminoacylation reaction), or by fusing the mutant cell line with a normal cell line, or a cell line mutant for a different tRNA synthetase (thereby complementing the mutant synthetase). The cell fusion experiments show that the aminoacyl-tRNA synthetase mutations are recessive at the cellular level. The chromosomal map positions of a number of human tRNA synthetases were first determined by analysis of (human) X (ts mutant CHO cell) hybrids. Human chromosomes are progressively lost in such hybrids, but one human chromosome - the one which contains the human synthetase complementary to the mutant hamster synthetase - is consistently retained. Such experiments provided the first evidence that

human tRNA synthetases are single copy genes in man (or at least confined to a single chromosome; refs. 6, 7). Subsequently Southern blotting and fluorescence *in situ* hybridization analyses have confirmed and extended these observations for thirteen synthetases (8-14). These Southern blotting and *in situ* hybridization mapping studies established beyond doubt that each of the human tRNA synthetase genes investigated is encoded at a single locus. The table below summarizes the chromosomal location of tRNA synthetases mapped to date.

Chromosome Location of tRNA Synthetases

tRNA synthase	Chromosome	tRNA Synthetase	Chromosome
Ala	16q22	Trp	14q21-32
Arg, Leu, His, Thr	5	Asp	2
Asn	18	Gln	3p
Cys	11p15.5	Gly	7
Glu/Pro	1q32-42	Ile (mitochondrial)	2
Gly	7p15	Lys	16q21
Ile	9q21	Ser	1p12
Lys	16q23-24	Tyr	1p31
Met	12	Val	6p21.3 9

Classification of tRNA synthetases

The twenty tRNA synthetases are divided into two groups based on structural features and functional properties that are conserved throughout evolution. There are ten class I synthetases, all of which contain two short conserved amino acid segments which fold together to form an ATP binding pocket called the Rossman fold, in the amino

terminal half of the proteins. The C-terminal end of the Class I synthetases contains the tRNA binding fold. Class II synthetases, of which there are also ten, share up to three short conserved amino acid motifs.

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Example 11: Sodium Potassium ATPase, 1 subunit (ATP1A1) - Target Gene VARIA125

Sodium Potassium ATPase is essential for cell survival

The plasma membranes of virtually all eukaryotic cells contain a Na+, K+ pump that operates as an antiport, pumping Na+ out of the cell and K+ in against their concentration gradients. In coupling the hydrolysis of ATP to the active transport of 3 Na+ out and 2 K+ into the cell the pump is electrogenic. The electrochemical gradients generated and maintained by the Na+,K+ pump are essential for the regulation of cell volume, and for the secondary, sodium-coupled active transport of a variety of organic and inorganic molecules including glucose, amino acids and Ca++. Hence the sodium potassium pump plays an essential role in cellular physiology (1).

Sodium Potassium ATPase is a heterodimer composed of a ~100 kDa catalytic subunit and a ~55 kDa glycoprotein subunit of unknown function. Biochemical studies and gene cloning have demonstrated the existence of three isoforms and two -like isoforms of the catalytic subunit, each encoded by a separate gene and with a characteristic expression pattern (reviewed in refs. 2 and 3). Of these, only the 1 gene

(ATP1A1) is ubiquitously expressed; the other subunits have restricted tissue distribution.

Sodium Potassium ATPase is the target of the cardiac glycoside drugs, including digoxin and the poison ouabain. Ouabain binds to the extracellular face of the 1 subunit and inhibits Na+,K+ exchange, leading to cell death. The 1 subunit from primates is sensitive to nanomolar concentrations of ouabain while the rodent 1 subunit is resistant to ~1000 fold higher concentrations, enabling precise definition of the ouabain binding site. Study of human-rat chimeric 1 subunits combined with site directed mutagenesis has localized the ouabain interacting domain in the amino-terminal portion of the 1 subunit (4,5). Other structure-function studies have contributed to an understanding of 1 subunit cation binding and ATPase functions, while electron microscopy and low resolution (20-30) diffraction analyses of membrane preparations have elucidated the geometry of the protein in the membrane (1).

The 1 subunit of Sodium Potassium ATPase has sequence variants

The cDNA sequence of the human 1 subunit of sodium-potassium ATPase has been published by four groups (6-9). We undertook a systematic search for DNA sequence variance by analyzing the 1 cDNA from 36 unrelated individuals using the single strand conformation polymorphism technique. Primers were designed using the sequence of Kawakami et al. (GENBANK accession D00099; see ref. 6). SSCP analysis revealed 7 sequence variances, and subsequent DNA sequence analysis confirmed that nucleotides 1059 (A *vs.* C), 1428 (G *vs.* A), 2538 (T *vs.* C), 3324 (C *vs.* T), 3375 (G *vs.* A), 3397 (G *vs.* A) and 3408 (C *vs.* A) vary as shown in the Target Summary Table. The first five sequence variances are in the coding sequence while the latter two are in the 3' untranslated region.

The frequency of heterozygotes for the seven sequence variants ranged from 3-11% among the 36 individuals tested. Some of the sequence variances appear to occur more commonly in certain racial or ethnic groups. For example, heterozygotes for four sequence variances (at nucleotides 1059, 1428, 3324 and 3375) were detected solely or predominantly in North American Blacks, with heterozygote frequencies of 1/4 or 2/4. The nucleotide 2538 variance was detected solely in North American Whites (4/16) and results in an amino acid exchange (see below). The nucleotide 3397 sequence variance was detected solely in one Japanese individual (of four tested). The nucleotide 2538 sequence variant results in an aspartic acid *vs.* glutamic acid substitution at amino acid 740 of the 1024 amino acid protein. This residue lies in the cytoplasmic loop of the 1 subunit.

The alpha1 subunit of Sodium Potassium ATPase maps to chromosome 1p13-p11

The gene for the 1 subunit of sodium-potassium ATPase has been mapped to chromosome band 1p13-p11 by several techniques. Yang-Feng et al. (10) assigned the ATP1A1 gene to 1p21-cen by Southern analysis of DNA from panels of rodent/human somatic cell hybrid lines. This localization was confirmed and refined by Chehab et al., who showed that the gene for the ATP1A1 subunit is on 1p13-p11 using hybridization to flow-sorted chromosomes and *in situ* hybridization (9).

Chromosome band 1p13-p11 is a site of frequent loss of heterozygosity

The short arm of chromosome 1 is comparatively well investigated for allele loss, especially in breast and colon cancers, however most of these studies are principally concerned with the 1p36 region, and there is comparatively little data on 1p13-p11. The best studies of proximal 1p allele loss are in breast and testicular cancers. These studies show LOH occurs in approximately 15-35% of breast cancers (11,12) and 15-25% of testicular cancers (13). Data from more distal loci on 1p show >25% LOH in

glioma, colon cancer, stomach cancer, ovarian cancer, and liver cancer (14). The LOH observed in this region indicates that other essential genes mapping to the 1p chromosomal arm, and especially to the 1p11 region, which have LOH and for which sequence variances, and therefore heterozygotes for a sequence variance, exist in normal somatic cells of individuals in a population are potential target genes

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Example 12: Ribonucleotide Reductase, M1 subunit (RRM1) - Target Gene VARIA200

Ribonucleotide Reductase is essential for cell growth

Human ribonucleotide reductase (also called ribonucleoside diphosphate reductase) is essential in dividing cells for the production of deoxyribonucleotides prior to DNA synthesis in S phase. Ribonucleotide reductase catalyzes the reduction of all four

ribonucleoside diphosphates to the corresponding deoxyribonucleoside diphosphates by replacing the 2' hydroxyl moiety of ribose with a hydride ion to form deoxyribose; these reactions constitute the first committed steps in the creation of DNA precursors (deoxyribonucleotides), and are therefore tightly regulated by allosteric nucleotide binding sites on the M1 subunit (2,3). The enzyme is an 2 2 tetramer apparently conserved in all prokaryotes and eukaryotes (1). The two subunits, M1 and M2, are both required for enzyme activity. The RRM2 subunit contains the catalytic site, while the RRM1 subunit provides an indispensable allosteric function. (See pages 758-763 of Biochemistry by C.K. Mathews and K.E. van Holde, Benjamin/Cummings Publishing Biochemistry, Company, Redwood City, 1990 for a fuller account of ribonucleotide reductase function.)

Both ribonucleotide reductase subunits are expressed in all proliferating cells but are generally nondetectable in quiescent cells. Ribonucleotide reductase subunit M2 is the target of several antineoplastic compounds, including hydroxyurea. Hydroxyurea is used in the chemotherapy of a variety of myeloproliferative disorders (4). It acts by reversibly destroying a tyrosyl free radical in the catalytic site of the M2 subunit (3). Hydroxyurea and other ribonucleotide reductase poisons are specific for the S phase of the cell cycle, resulting in growth arrest at the G1-S boundary and apoptotic death in tumor cells (5). Exposure of cell cultures to hydroxyurea results in selection of cells expressing high levels ribonucleotide reductase, demonstrating that ribonucleotide reductase is required for these cells to grow (6).

The human ribonucleotide reductase gene has sequence variances

The cDNA sequence of the human ribonucleotide reductase M1 subunit has been published by two groups (7,8). We undertook a systematic search for DNA sequence variance in the cDNA of the M1 subunit by analysing 36 unrelated individuals using the single strand conformation polymorphism technique. Primers were designed using

the sequence of Parker et al. (GENBANK accession X59543; see ref. 7). SSCP analysis revealed 4 sequence variances, and subsequent DNA sequence analysis confirmed that nucleotides 1037 (C *vs.* A), 2410 (A *vs.* G), 2419 (A *vs.* G) and 2717 (T *vs.* A) vary as shown in the Target Summary Table. (The sequence variance at nt 1037 was previously noted by Parker et al., ref. 7.) Also, DNA sequencing revealed an insertion/deletion sequence variance: the 9 consecutive T nucleotides between positions 2724 and 2732 (numbering from ref. 7) were augmented in some cDNAs by a tenth T. (This sequence variance is designated T9 *vs.* T10 in the Target Summary Table.)

Both alleles at nt 1037 were detected in North American Whites, Hispanics, Chinese, Japanese, Arabs and Indians. Similarly, both alleles of the sequence variance at nt 2410 were detected in virtually all tested populations: North American White, North American Black, Hispanic, Chinese, Arab and Indian. In contrast, the sequence variances at nt 2419 and 2717 were prevalent in North American Blacks, Hispanics, Chinese, and Japanese, but not North American Whites. The insertion/deletion sequence variance at nt 2724 was only studied in four individuals so no firm conclusions can be drawn regarding population distribution, however it appears to be in linkage disequilibrium with the 2419 and 2724 sequence variances.

The human ribonucleotide reductase gene maps to chromosome 11p15.5

The gene for human ribonucleotide reductase has been mapped to band 11p15.5 by several techniques. Initially the gene was localized by Southern hybridization analysis of human X rodent somatic cell hybrids and by chromosomal *in situ* hybridization (9). Subsequently RRM1 has been placed on a yeast artificial chromosome (YAC) physical map of chromosome 11p15 (10). The precise physical localization of the RRM1 gene facilitates interpretation of LOH results at adjacent polymorphic markers (see below).

Chromosome band 11p15.5 is a site of frequent loss of heterozygosity

The short arm of chromosome 11 is the site of several tumor suppressor genes, including the WT1 gene and the Beckwith-Weidemann syndrome gene. As a result there are many studies of LOH in 11p15.5, particularly focusing on breast, cervix, kidney, liver, lung, ovarian, stomach and testicular cancers. These studies show that the 11p15.5 band of chromosome 11 is frequently reduced to one copy (11-28). For example, LOH occurs in approximately 13-33% of breast cancers (11-13), 14-42% of cervical cancers (14), 0-50% of liver cancers (16), 0-80% of lung cancers (17-19), 18-54% of ovarian cancers (20,21), 0-71% of stomach cancers (22) and 0-50% of testicular cancers (23,24). Other studies show that 11p15.5 LOH may also be frequent in bladder cancer (25), esophageal cancer (26), some leukemias (27) and sarcomas (28). Many deletions in the 11p15.5 region span relatively short chromosomal segments (2 - 10 megabases; see ref. 17).

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Example 13: Thymidylate Synthase (TS) - Target Gene VARIA250

Thymidylate Synthase is essential for cell growth

Human thymidylate synthase (TS) catalyzes the formation of thymidine monophosphate (dTMP) from deoxyuridine monophosphate (dUMP) by transfer of a methyl group from N5,N10-methylenetetrahydrofolate to carbon 5 of dUMP. This is the sole *de novo* pathway to dTMP, an essential precursor for DNA synthesis. TS also plays an important role in balancing the four nucleotide precursors for DNA polymer synthesis (1). Thus TS is an attractive target for antiproliferative drugs. (*See* Biochemistry by C.K. Mathews and K.E. van Holde, Benjamin/Cummings Publishing Company, Redwood City, 1990, pages 763-768, for a fuller account of thymidylate synthase function.)

Like some other growth associated genes involved in DNA synthesis, thymidylate synthase is expressed in proliferating cells at 20-40 fold higher levels than in quiescent cells. Increased expression occurs at the G1-S transition of the cell cycle when quiescent cells are stimulated with serum. Levels of thymidylate synthase are finely controlled by autoregulatory feedback loops wherein TS protein regulates the transcription, stability and translational efficiency of TS mRNA (2). Transcription increases by only 2-4 fold, so posttranscriptional events constitute the predominant regulatory mechanisms (3). One mechanism of 5-FU resistance is increased expression of TS Mrna.

Thymidylate synthase is the target of 5-fluorouracil (5-FU), a potent antineoplastic compound. Once inside cells 5-FU is ribosylated and phosphorylated to 5-fluoro-2'-deoxyuridine 5'-monophosphate (F-dUMP), which acts as an inhibitory transition state analog of TS when bound in the presence of the enzyme's second substrate, N5,N10-methylenetetrahydrofolate. (5-FU is also incorporated into both DNA and RNA,

augmenting its toxicity.) 5-FU induces partial responses in 10-30% of patients with a variety of cancers, including metastatic breast and gastrointestinal tract cancers (4). While 5-FU is a potent antiproliferative agent in tissue culture cells, as with most antineoplastic drugs, its clinical utility is limited by lack of discrimination between normal cells and tumor cells: common toxic effects include stomatitis, diarrhea, bone marrow suppression, hair loss and occasionally cardiac and neurologic symptoms.

The human thymidylate synthase gene has sequence variances

The sequence of a human thymidylate synthase cDNA was determined by Takeishi et al. (5), who later determined the genomic sequence as well (6). We undertook a systematic search for DNA sequence variance by analysing 36 unrelated individuals using the single strand conformation polymorphism. Primers were designed using the sequence of Takeishi et al. (5). SSCP analysis revealed 3 DNA fragments having sequence variances, and subsequent DNA sequence analysis showed that nucleotides 1066 (C *vs.* T), 1136 (A *vs.* G) and 1497 (A *vs.* T) vary among normal individuals as shown in the Target Summary Table. All three sequence variances are in the 3' untranslated region of the gene. The nucleotide 1066 and 1497 sequence variances are in complete linkage disequilibrium in the 36 individuals examined. Both alleles of all three sequence variances were detected in North American Whites, North American Blacks, Chinese, Japanese, Arabs and Indians.

Another TS sequence variance has been described by Berger and colleagues (7-9). They detected a T to C change at nucleotide 276 of the TS gene, resulting in the substitution of histidine for an evolutionarily conserved tyrosine at residue 33 of TS protein. So far the histidine allele has been detected in only one cell line, HCT116 (7). The rare his-33 form of the protein is 3-4 fold more resistant to FdUrd than the tyr-33 form, due to an 8 fold lower catalytic efficiency (kcat), suggesting that histidine at residue 33 perturbs the structure of the TS active site (9)

The human thymidylate synthase gene maps to chromosome 18p11.32

The gene for human thymidylate synthase was initially mapped to the long arm of chromosome 18 (18q21.31-qter) by somatic cell hybrid analysis (10), however two subsequent reports place the gene in band 18p11.32 using fluorescence *in situ* hybridization (11,12).

Chromosome band 18p11.32 is a site of loss of heterozygosity

The long arm of chromosome 18 contains the DCC (deleted in colon cancer) candidate tumor suppressor gene and has been well studied in a variety of tumors. The short arm (18p), where TS apparently resides, has not been studied as extensively. The available data suggests there is LOH in approximately 45% of colon cancers (13) and 25-30% of cervical (14), head and neck (15), lung (16) and ovarian (17) cancers and sarcomas. LOH has also been described in breast, brain, esophagus, kidney and prostate cancers (0-15%). 18p has not been studied for allele loss in several other major cancers, including bladder, leukemia, lymphoma, liver, pancreas, stomach and testicular cancers.

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Example 14: Cytidine Triphosphate Synthetase (CTPS) - Target Gene VARIA260

Cytidine Triphosphate Synthetase is essential for cell growth

Human cytidine triphosphate synthetase catalyzes the glutamination of UTP to form CTP. The reaction is: UTP + ATP + glutamine --> CTP + ADP + P*i* + glutamate. This is the rate limiting step in the synthesis of cytidine nucleotides from both the *de novo* and uridine salvage synthesis routes (see ref. 1 and references therein). CTPS also plays a vital regulatory function in balancing nucleotide pools for DNA polymer synthesis; it is allosterically regulated by CTP (negatively) and GTP (positively).

There is compelling evidence that CTPS is essential for cell survival:

CTPS is evolutionarily conserved in yeast and bacteria, with a high degree of amino acid identity in regions mediating allosteric regulation and catalysis (1-

3). (Another example: the human and hamster enzymes are identical in length and 98% amino acid identical over 591 amino acids.)

Mutant hamster cells lacking functional CTPS need exogenous cytidine to survive (3).

There is no known human deficiency disease of CTPS.

CTPS function is increased in proliferating cells (4).

Thus CTPS is an attractive target for antiproliferative drugs. Cyclopentyl cytosine (CPE-C) is a synthetic cytidine analog in which a cyclopentyl group replaces the furan ring of the ribose sugar. CPE-C has antineoplastic and antiviral effects in animal models (5). The drug is kinased intracellularly to the triphosphorylated nucleotide form (CPE-CTP). Exposure of cells to CPE-C leads to rapid depletion of CTP pools, as a result of inhibition of CTPS by CPE-CTP (6,7). Upregulation of CTP synthetase, or loss of negative allosteric modulation by CTP is associated with resistance to the cancer chemotherapy drugs arabinosyl cytosine (ara-C), 5-fluorouracil and other cytotoxic nucleoside analogs as well as alkylating agents (3).

The human cytidine triphosphate synthetase gene has sequence variances

The sequence of a human cytidine triphosphate synthetase cDNA was determined by Yamauchi et al. (1), who later determined the genomic sequence as well (2). We undertook a systematic search for DNA sequence variance by analysing 36 unrelated individuals using the single strand conformation polymorphism technique. Primers were designed using the sequence of Yamauchi et al. (1). SSCP analysis revealed 3 DNA fragments having sequence variances, and subsequent DNA sequence analysis showed that nucleotides 576 (A *vs.* G), 2093 (C *vs.*T) and 2135 (G *vs.* A) vary among normal individuals as shown in the Target Summary Table. The nucleotide 576 sequence variance is a silent substitution in the coding region, while the latter two sequence variances are in the 3' untranslated region of the cDNA. All three sequence

variances were detected at low frequency in the panel of 36 individuals (3-8%), however all but one of the heterozygotes is Asian, and it seems likely that a larger survey of Asian populations would show higher allele frequencies in Chinese and other groups. For example among the four Chinese in the panel two (50%) are heterozygous for the residue 2135 sequence variance, and one (25%) is heterozygous for the nt 576 sequence variance. Also, the one Cambodian in the panel is heterozygous for both the 2093 and 2135 sequence variances.

The human cytidine triphosphate synthetase gene maps to chromosome 1p34.1

The gene for human cytidine triphosphate synthetase has been mapped to 1p34.1 by somatic cell hybrid analysis (2).

Chromosome band 1p34.1 is a site of frequent loss of heterozygosity

The short arm of chromosome 1 is comparatively well investigated for allele loss, especially in breast and colon cancers. The 1p35-32 and 1p22-13 regions flank 1p34.1 and are the best available markers for LOH on 1p. Studies of these regions show 30-50% LOH frequency in breast cancer (8-12), 41-75% in glioma (a brain cancer subtype) (13), 20-40% in colon cancer (14,15), ~50% in stomach cancer (16), ~20% in lung cancer (17) and 20-30% in ovarian cancer (18). High frequency LOH has been detected in several uncommon cancers such as pheochromocytoma (50-86%) and neuroblastoma (~50%). Most other common cancers have not been adequately investigated to assess LOH frequency in this region.

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Example 15: Cysteinyl tRNA Synthetase (CARS) - Target Gene VARIA301

The human cysteinyl tRNA synthetase gene is essential for cell survival

Cysteinyl-tRNA synthetase (CARS) catalyzes ATP dependent covalent attachment of

cysteine to its cognate tRNA to form cysteinyl-tRNA. In the absence of cysteinyl-tRNA, protein synthesis is blocked. Since Cysteinyl-tRNA synthetase is a single copy gene in man, inhibition of its function is expected to be cell lethal. This has been shown for other tRNA synthetases (summarized above).

The human cysteinyl-tRNA synthetase gene and mRNA have sequences variances

A human cDNA encoding cysteinyl tRNA synthetase (CARS) was cloned based on the similarity of a human expressed sequence tag (EST) to *E. coli* cysteinyl tRNA synthetase (1). The published human CARS cDNA is 2048 nucleotides in length and includes a 30 nucleotide 5' untranslated region followed by an open reading frame of 1914 nucleotides and a 3' untranslated region of 134 nucleotides (1). An EMBL/ GENBANK submission (accession # L06845) by the authors of ref. 1 includes a 3' untranslated region 423 nucleotides longer than the published sequence, but lacks 19 consecutive A nucleotides after position 2029 (making a net increase of: 423 - 19 = 404 nucleotides, and a composite cDNA of: 2048 + 404 = 2452 nucleotides in length. We have confirmed the existence of 2452 nt transcripts by PCR amplification of reverse transcribed mRNA.) We designed primers as shown on the annotated cDNA sequence and screened the composite 2452 nt cDNA for sequence variance in 36 unrelated individuals by the single strand conformation polymorphism (SSCP) technique. Two sequence variances were identified. One of the sequence variances, located in the 5' untranslated region, was below the desired level of 20% heterozygosity. The other sequence variance is a C *vs.* T transition near the 3' end of the coding sequence at nucleotide 1739 (see annotated sequence).

The human cysteinyl tRNA synthetase protein has sequence variances

The deduced amino acid sequence of the human CARS gene encodes a protein of 638 amino acids which probably functions as a monomer, by analogy to related synthetases. The deduced protein contains two sequence motifs, HIGH (residues 64-

67) and KMSKS (residues 406-410), which define Class I synthetases (see ref. 2 for background information on tRNA synthetases). These two conserved motifs form an ATP binding fold (the Rossman fold) in the amino terminal half of the protein. Cytosine at nucleotide 1739 encodes proline at residue 622 of the protein, while thymine at nucleotide 1739 encodes leucine. The pro/leu amino acid sequence variance is a mere 16 residues from the C terminus of the protein. The C-terminal portion of CARS, by analogy to other class I synthetases, contains the tRNA binding site.

Frequency of CARS heterozygotes

The frequency of heterozygotes for the nucleotide 1739 sequence variance is ~45-50% in all major racial groups surveyed (see accompanying table), including North American Whites (8/15=53%), North American Blacks (2/4=50%), Chinese (2/4=50%), Swedish (127/344=37%) and Japanese (1/4=25%). The wide population distribution of both alleles suggests that other population groups will also have a high frequency of heterozygotes.

Gene Mapping of CARS to 11p15.5

Human CARS cDNA has been mapped to chromosome 11p15.5 by screening human X Chinese hamster somatic cell hybrids informative for all human chromosomes, and by fluorescence *in situ* hybridization (3). Both mapping techniques were conclusive in showing only one locus for human CARS. Detailed physical maps of 11p15.5 have subsequently allowed precise localization of the CARS gene relative to other DNA markers (4).

LOH at 11p15.5 is well documented in many cancer types

The short arm of chromosome 11, and particularly the 11p15.5 region, is deleted in a

variety of human cancers, including (but not limited to) ovarian (18 - 50% LOH), non-small cell lung (22 - 71%), breast (12 - 33%), bladder (40 -50%), esophageal (18 - 40%) and testicular cancers (18 - 66%) (refs. 5-12). Many deletions in the 11p15.5 region span relatively short chromosomal segments (2 - 10 megabases; see ref. 8). Using the specific variances identified in the CARS gene as markers for heterozygosity, we have determined that LOH occurs in 10/20 ovarian cancers (50%) and 10/52 non-small cell lung cancers (19%).

Assays for human CARS inhibitors

There is no published work on the protein encoded by the putative human CARS cDNA, nor on any other eukaryotic CARS protein, however the extensive characterization of other Class I synthetases from both prokaryotes and eukaryotes provides a template for modeling the structure of human CARS. (For an example of how this can be done see ref. 14, in which the three dimensional structure of human alanyl-tRNA synthetase has been modeled up to amino 249 by neural net software and multiple alignments of partial and complete human AARS sequences with heterologous prokaryotic class II synthetases for which crystal structures exist.) With respect to the C-terminal location of the variant amino acid residue in human CARS, it is worth noting that single amino acid substitutions in the C-terminal region of alanyl tRNA synthetase can have greater than 100 fold effects on catalytic activity (15).

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Example 16: Glutamyl-Prolyl tRNA Synthetase (EPRS): - Target Gene VARIA300

The human glutamyl-prolyl tRNA synthetase gene is essential for cell survival

Glutamyl-prolyl-tRNA synthetase (EPRS) catalyzes ATP dependent covalent attachment of glutamine and proline to their cognate tRNAs to form glutamyl-tRNA and prolyl-tRNA. In the absence of glutamyl-tRNA *or* prolyl-tRNA, protein synthesis is blocked. Since glutamyl-prolyl-tRNA synthetase is a single copy gene in man, inhibition of its function is expected to be cell lethal. This has been shown for other tRNA synthetases (summarized above).

The human glutamyl-prolyl tRNA synthetase gene, mRNA and protein have sequence variances

A human cDNA encoding glutamyl-prolyl tRNA synthetase (EPRS) was initially misidentified as glutaminyl-tRNA synthetase (1) based on misleading sequence alignments with bacterial and yeast glutaminyl-tRNA synthetase (2). Subsequently, biochemical studies of the protein encoded by a *D. melanogaster* gene ~70% identical to the human gene demonstrated glutamyl (not glutaminyl) tRNA synthetase activity, and also showed that a single gene encodes both glutamyl- and prolyl-tRNA synthetases in the fly (3). These observations eventually led to the realization that

human EPRS is also a single polypeptide containing two synthetases (2). The aminoacyl tRNA synthetases are divided into two classes (see *Background on tRNA Synthetases*, above). Glutamyl-tRNA synthetase belongs to Class I while Prolyl-tRNA synthetase belongs to class II. Thus the two halves of EPRS evolved independently and likely represent an evolutionarily recent fusion. The published human EPRS cDNA is 4,586 nt long and includes a 5' untranslated region of 58 nt followed by an open reading frame of 4320 nt and a 3' untranslated sequence of 208 nt (1). The gene encodes a polypeptide of 1440 amino acids. The glutamyl-tRNA synthetase activity is encoded by an imprecisely defined segment at 5' end of the gene probably spanning at least amino acids 105-426, while the prolyl-tRNA synthetase activity is encoded by a segment likely including residues 942-1369 at the 3' end of the gene (2). The two synthetase moieties are connected by a central domain of unknown function. It has been speculated that the central domain may attach the enzyme to the cytoskeleton or to other aminoacyl-tRNA synthetases in a multienzyme complex (2, 3).

The human glutamyl-prolyl-tRNA synthetase gene and mRNA have sequence variances

We designed primers and screened the 4586 nt cDNA for sequence variance in 36 unrelated individuals by the single strand conformation polymorphism technique. Seven sequence variances were identified, four located in the coding sequence and three located in the 3' untranslated region. As shown on the Annotated Glutamyl-Prolyl tRNA Synthetase cDNA Sequence and in the Target Summary Page, the sequence variance nucleotides are 2520 (C *vs.* A), 2944 (G *vs.* A), 2963 (C *vs.* T), 2969 (A *vs.* G), 3247 (A *vs.* G), 4459 (G *vs.* A) and 4506 (G *vs.* A). The sequences flanking the alternate allelic forms and their frequencies of occurrence are shown on the Target Summary Page. Less than 10% of individuals surveyed are heterozygous for sequence variances at 2520, 2944 and 2963. Heterozygotes for the other 4 sequence variances occur more frequently and appear to be widely distributed in the surveyed populations (see below).

The human glutamyl-prolyl tRNA synthetase protein has sequence variances Three nucleotide sequence variances, at 2520, 2963 and 2969, alter the amino acid coding sequence of EPRS at residues 821 (pro/his), 969 (his/tyr) and 971 (ile/val). The residue 821 his and 969 tyr alleles are relatively rare, with fewer than 10% heterozygotes in the surveyed populations. The more common residue 971 sequence variance lies in the PRS domain of the protein, near one of the widely conserved defining motifs for class II tRNA synthetases.

EPRS heterozygotes are frequent in non-Asian populations. While the overall frequency of residue 971 heterozygotes is 8/36 (24%), the frequency of heterozygotes varies among different populations. For example, there are no heterozygotes among 10 Asians surveyed (Chinese, Japanese, Filipino and Korean), while 8/26 (31%) of non-Asians, including North American Whites, Blacks and Hispanics, are heterozygotes.

The EPRS Gene Maps to 1q41-q42

Human EPRS cDNA has been mapped to chromosome 1q41-42 by screening human X Chinese hamster somatic cell hybrids informative for all human chromosomes, and by fluorescence *in situ* hybridization (3). Both mapping techniques were conclusive in showing only one locus for human EPRS.

Loss of heterozygosity at 1q41-42 is documented in several cancer types. 17-25% of breast cancers have allele loss in the 1q41-q42 region (4, 5), 29-46% of colon cancers (6, 7) and 17-26% of cervical cancers (8). One report describes 27% LOH in stomach cancer (9). One or two studies of brain, esophageal, kidney, liver and ovarian cancers also report LOH. No studies of LOH in the 1q41-q42 region have been reported in bladder, endocrine, head and neck, lung, or pancreas cancers or in leukemia or lymphoma.

Antisense considerations The sequence variances at 2963 and 2969 are close enough that a 20-mer antisense oligonucleotide could easily span them. Such an oligonucleotide should afford greater allele discrimination than is possible with a single nucleotide difference. However, the 2963 sequence variance is fairly rare (<10% heterozygotes) and not in linkage disequilibrium with the 2963 sequence variance, so there are more than two haplotypes in the populations tested.

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Example 17: Alanyl-tRNA Synthetase (AARS) - Target Gene VARIA304

The human glutamyl-prolyl tRNA synthetase gene is essential for cell survival

Alanyl-tRNA synthetase (AARS) catalyzes ATP dependent covalent attachment of alanine to its cognate tRNA to form alanyl-tRNA. In the absence of alanyl-tRNA, protein synthesis is blocked. Since alanyl-tRNA synthetase is a single copy gene in man (see below) inhibition of its function is expected to be cell lethal. This has been shown for other tRNA synthetases (summarized above).

The human alanyl-tRNA synthetase gene and mRNA have sequence variances

A human cDNA encoding alanyl tRNA synthetase (AARS) was cloned by Shiba et al. (1) using cross species PCR: AARS sequences from four evolutionarily distant species were compared and primers were designed to conserved regions specific to AARS. The cloned human cDNA is 3344 nt in length and includes a 110 nt 5' untranslated region, an open reading frame of 2904 nt encoding a 968 residue polypeptide, and a 3' untranslated region of 330 nt (ref. 1; Genbank accession D32050).

We designed primers. The 3344 nt cDNA was screened for sequence variance in 36 unrelated individuals by the single strand conformation polymorphism (SSCP) technique. One sequence variance was identified, a C *vs.* T transition at nucleotide 1013, within the coding sequence. The published nucleotide at position 1013 is T (1).

The frequency of AARS heterozygotes is 25-50% in all populations surveyed. The frequency of heterozygotes for the nucleotide 1013 sequence variance is 57% in the 36 individuals tested. Both alleles are present in all major racial groups surveyed (see Target Gene Summary Table), including North American Whites (9/15=60% heterozygotes), North American Blacks (3/4=75%), Chinese (2/4=50%), Japanese (1/4=25%) and Hispanic (1/2). The wide population distribution of both alleles suggests that other population groups will also have a high frequency of heterozygotes.

The AARS gene maps to 16q22

The human AARS cDNA has been mapped to chromosome 16q22 by us and by Nichols et al. (ref. 2). We designed primers to the 3' untranslated region of AARS and used PCR to analyze the National Institute of General Medical Sciences (NIGMS) Human/Rodent Somatic Cell Hybrid Mapping Panel #2 (see page 704 of the NIGMS 1994/1995 Catalog of Cell Lines, available from the Coriell Cell Repository, Camden, NJ). The panel consists of 24 hybrid cell lines, each monochromosomal for one human chromosome. The AARS PCR product mapped to the hybrid containing human chromosome 16. Subsequently we screened the Radiation Hybrid Mapping Panel created at Stanford University (rhserver@shgc.stanford.edu) and distributed by Research Genetics (RH01). The AARS PCR product mapped near D16S496 with a lod score>10. D16S496 is a polymorphic DNA marker at 16q22. The AARS PCR product mapped near D16S496 with a LOD score >10. DH16S496 is a polymorphic DNA marker at 16q22. (See, ref. 29 for a full explanation of modification hybrid mapping.) Similar results were obtained by Nichols et al., who mapped AARS by analysis of the same NIGMS hybrid mapping panel, by PCR mapping in a chromosome 16 regional mapping panel and by fluorescence *in situ* hybridization to metaphase chromosomes. All mapping techniques were conclusive in showing only one locus for human AARS.

LOH at 16q22 is well documented in many cancer types. Loss of heterozygosity studies of chromosome 16q have principally focused on breast and liver cancers. In six detailed studies of breast cancer in the 16q22 region LOH frequencies of 40-60% have been reported (refs 3-8). 16q22 LOH has ben reported in 25-90% of liver cancers (9-13), with the average around 45%. Less extensive studies of other cancer types report 16q22 LOH in 19% of bladder cancers, 20% of colon cancers (14), 19-27% of esophageal cancers (15), 25% of small cell lung cancers (16), 16-37% of ovarian cancers (17-19) and 22% of uterine cancers (20), and 31-50% of prostate cancers (21-

22).

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Example 18: Threonyl-tRNA Synthetase (TARS) - Target Gene VARIA302

The human threonyl-tRNA synthetase gene is essential for cell survival

Threonyl-tRNA synthetase (TARS) catalyzes ATP dependent covalent attachment of threonine to its cognate tRNA to form threonyl-tRNA. In the absence of threonyl-tRNA, protein synthesis is blocked. Threonyl-tRNA synthetase is a single copy gene in man (see below) and inhibition of TARS is cell lethal. This has been shown using the specific TARS inhibitor borrelidin, a threonine analog. Borrelidin resistant CHO cell lines have been isolated; the most resistant lines contain ~60-100 fold more immunologically reactive protein and 10-20 fold higher TARS activity than non-selected CHO cells (1-3).

The human TARS enzyme is a homodimeric member of the class II tRNA synthetases. The human protein is 53% amino acid identical to *S. cerevisiae* cytoplasmic TARS, 40% amino acid identical to *E. coli* TARS and 39% amino acid identical to yeast mitochondrial TARS. The degree of evolutionary conservation is 52-64% when conservative substitutions are allowed.

The human Threonyl-tRNA synthetase gene and mRNA have sequence variances. A human cDNA encoding threonyl tRNA synthetase was cloned by Cruzen and Arfin (GENBANK accession M63180; ref. 2) using anti-TARS antibodies to screen a lgt11 expression library. The cDNA is 2644 nt in length and includes a 138 nt 5' untranslated region, an open reading frame of 2136 nt encoding a 712 residue polypeptide, and a 3'

untranslated region of 370 nt.

We designed primers for amplification. The 2644 nt cDNA was screened for sequence variance in 36 unrelated individuals by the single strand conformation polymorphism (SSCP) technique. Three sequence variances were identified: G *vs.* A transitions at nucleotides 1608 and 1755 within the coding sequence, and a C *vs.* T transition at nucleotide 2395 of the 3' untranslated region. None of the sequence variances alters the sense of the coding strand. The published sequence shows G, G and T at the three sequence variance sites

The frequency of TARS heterozygotes is 25-45% in all populations surveyed. The nucleotide 1608 sequence variance was genotyped only in North American Whites, 45% of whom were heterozygotes. The nucleotide 1608 and 1755 sequence variances were both genotyped in 36 individuals, with overall heterozygosity rates of 31% and 25%, respectively. Both sequence variances were detected in North American Whites, North American Blacks, Hispanics and Chinese. Of 14 North American Whites genotyped at all 3 sequence variance nucleotides, 11 (79%) were heterozygous for a least one polymor-phism (see threonyl tRNA synthetase summary table).

The TARS gene maps to 5p13-CEN. The human TARS cDNA has been mapped to chromosome 5p13-CEN by analysis of TARS isoelectric focusing patterns in human/Chinese hamster hybrids (). The mapping studies were consistent with one human TARS locus.

LOH at 5p13-CEN is documented in several cancer types. The best data on 5p LOH is in cervical cancer where 9 markers have been tested in 3 different studies. The frequency of LOH ranges from 12-57%, averaging ~45%. Other cancers that have been studied are breast (10-24% LOH), head and neck (20% LOH), adenocarcinoma of the lung (40% LOH, but only 5 cancers were studied), melanoma (40%) and ovary (15-

21%).

Assays for human TARS inhibitors. Human TARS protein is a homodimeric class II synthetase. Antibodies to rat TARS were used to clone the human protein. The high degree of amino acid conservation throughout the protein suggests that it may be possible to create yeast and/or bacterial strains with human CARS.

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Example 19: Glutaminyl-tRNA Synthetase (QARS) - Target Gene VARIA305

The human glutaminyl-tRNA synthetase gene is essential for cell survival

Glutaminyl-tRNA synthetase (QARS) catalyzes ATP dependent covalent attachment of glutamine to its cognate tRNA to form glutaminyl-tRNA. In the absence of glutaminyl-tRNA, protein synthesis is blocked in eucaryotic cells. Glutaminyl-tRNA synthetase is a single copy gene in man . Inhibition of its function is expected to be cell lethal, as shown for other tRNA synthetases (summarized above).

The human Glutaminyl-tRNA synthetase gene and mRNA have sequence variances.

A human cDNA encoding glutaminyl tRNA synthetase (QARS) was cloned by Lamour et al. (1) who expressed the cDNA in *E. coli* and demonstrated glutaminyl tRNA synthetase activity in bacterial extracts. The cloned human cDNA

(Genbank/EMBL accession number X76013) is 2437 nt in length and includes a 5' untranslated region of 5 nucleotides, an open reading frame of 2325 nucleotides encoding a 775 amino acid polypeptide, and a 3' untranslated region of 107 nt including 8 terminal nt of poly A.

We designed primers for amplification. The QARS cDNA was screened for sequence variance in 36 unrelated individuals using the single strand conformation polymorphism (SSCP) technique. One sequence variance was identified, a C *vs.* T transition at nucleotide 404, within the coding sequence. The published nucleotide at position 404 is C. The sequence variance does not affect the protein encoded.

The frequency of heterozygotes for the nucleotide 404 sequence variance is 11% in the 36 individuals tested (4/36). However three of 16 North American Whites are heterozygotes (19%), and one of four Japanese (25%) (see Target Gene Summary Table).

The QARS gene maps to 3p

The human QARS cDNA has been mapped to chromosome 3 by hybridization of a QARS probe to a panel of 25 human/rodent somatic cell hybrids (1). One somatic cell hybrid, not known to contain human chromosome 3, was positive for both the QARS probe and an ACY1 probe. ACY1 maps to human 3p21, suggesting QARS may also map in this area. We independently mapped QARS to chromosome 3 using primers from the 3' untranslated region to analyze the National Institute of General Medical Sciences (NIGMS) Human/Rodent Somatic Cell Hybrid Mapping Panel #2 by PCR (see page 704 of the NIGMS 1994/1995 Catalog of Cell Lines, available from the Coriell Cell Repository, Camden, NJ). The panel consists of 24 hybrid cell lines, each monochromosomal for one human chromosome. The QARS PCR product mapped to the hybrid containing human chromosome 3. All mapping techniques were conclusive

in showing only one locus for human QARS.

Chromosome band 3p21 is a site of frequent loss of heterozygosity. The short arm of chromosome 3 has been well studied in breast, cervical, esophageal, kidney, and lung cancers. These studies report frequent allele loss at 3p21, varying up to 100% in some studies of small cell lung cancer. Among other cancers LOH occurs in approximately 20-30% of breast cancers (2,3), 30-60% of cervical cancers (4,5), 10-40% of esophageal cancers (6,7), 45-80% of kidney cancers (8-10), 50-100% of nasopharyngeal cancers (11), 0-75% of squamous cell head and neck cancers (12), 30-60% of melanomas (13), 30-100% of non-small cell lung cancers (14-16) and 80-100% in small cell lung cancer (17-19). Other for which there are reports of LOH in at least 20% of cases include leukemia, pancreas cancer, sarcoma, testis cancer and ovarian cancer. Other cancer types, including bladder and lymphoma, have not been studied for LOH at 3p21.

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Example 20: Lysyl-tRNA Synthetase (KARS) - Target Gene VARIA303

Human Lysyl t-RNA synthase gene is essential

Lysyl-tRNA synthetase (KARS) catalyzes ATP dependent covalent attachment of lysine to its cognate tRNA to form lysyl-tRNA. In the absence of lysyl-tRNA, protein synthesis is blocked. Since lysyl-tRNA synthetase is a single copy gene in man, inhibition of its function is expected to be cell lethal. This has been shown for other tRNA synthetases (summarized above).

The human Lysyl-tRNA synthetase gene and mRNA have sequence variances

A human cDNA encoding a sequence similar to bacterial lysyl tRNA synthetases was cloned by Nomura et al. (GenBank/DDBJ submission D31890; see ref. 1) while sequencing random cDNAs. No biochemical studies of the protein encoded by this sequence have been reported. The 5' end of the sequence apparently begins in the coding region and the open reading frame continues for 1805 nucleotides, encoding 601 residues of a polypeptide (the full length of which has not been established), followed by a 3' untranslated region of 165 nucleotides.

We designed primers for amplification. The reported partial cDNA was screened for sequence variance in 36 unrelated individuals using the single strand conformation polymorphism (SSCP) technique as described in the methods section. Two sequence variances were identified, an A *vs.* G transition at nucleotide 89 and a G *vs.* C transversion at nucleotide 1798, both within the coding sequence. The published nucleotides are A and G, respectively. The nucleotide 1798 sequence variance alters the sense of the 599th codon (the third codon from the end of the coding sequence) to serine *vs.* threonine.

The frequency of KARS heterozygotes varies among the populations surveyed. The frequency of heterozygotes for the nucleotide 89 sequence variance is 19% in the 36 individuals tested. However all heterozygous individuals were either North American Whites (4/16; 25% heterozygotes), North American Blacks (1/4; 25%), or Hispanics (1/3; 33% heterozygotes). The frequency of heterozygotes for the nucleotide 1798 sequence variance is 6% in the 36 individuals tested. However all heterozygous individuals were North American Blacks (2/4; 50%) (see Target Gene Summary Table). Further study of these and other population groups will better establish the frequency of heterozygotes for these two sequence variances.

The KARS gene maps to 16q23-q24

The human KARS cDNA has been mapped to chromosome 16q22 by Nichols et al. (ref. 2) and by us. We designed primers to the 3' untranslated region of KARS and used PCR to analyze the National Institute of General Medical Sciences (NIGMS) Human/Rodent Somatic Cell Hybrid Mapping Panel #2 (see page 704 of the NIGMS 1994/1995 Catalog of Cell Lines, available from the Coriell Cell Repository, Camden, NJ). The panel consists of 24 hybrid cell lines, each monochromosomal for one human chromosome. The KARS PCR product mapped to the hybrid containing human chromosome 16. Similar results were obtained by Nichols et al., who mapped KARS

by analysis of the same NIGMS hybrid mapping panel, by PCR mapping in a chromosome 16 regional mapping panel and by fluorescence *in situ* hybridization to metaphase chromosomes. The *in situ* hybridization showed KARS maps to 16q23-q24. All mapping techniques were conclusive in showing only one locus for human KARS.

Loss of heterozygosity occurs frequently at 16q23-q24 in many cancer types. Loss of heterozygosity studies of chromosome 16q have principally focused on breast and liver cancers. In six detailed studies of breast cancer in the 16q23-q24 region LOH frequencies of 30-60% have been reported (refs 3-8). 16q22 LOH has ben reported in 35-65% of liver cancers (9-13), with the average around 45%. Studies of other cancer types report 16q22 LOH in 19% of colon cancers (14), 17-27% of esophageal cancers (15,16), 37% of ovarian cancers (new ref) (17-19), 18% of prostate cancers (20) and 23% of uterine cancers (21). Cancer types not yet investigated for LOH include kidney, leukemia and lymphoma, lung, melanoma, neuroblastoma, stomach and testis.

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Example 21: Ribosomal Protein S14 (RPS14) - Target Gene VARIA326

Ribosomal protein S14 is essential for cell growth

Human ribosomal protein S14 (RPS14) is one of ~80 unique protein constituents of the mammalian ribosome. Many of the protein subunits of ribosomes, the protein making machines of all cells, are highly conserved throughout prokaryotic and eukaryotic evolution (1). For example, human RPS14 protein is 100% amino acid identical to hamster S14 protein, 72% identical to yeast rp59 protein and 43% identical to *E. Coli* ribosomal protein S11 (2,3). Mammalian S14 and yeast rp59 are components of the 40S ribosomal subunit while *E. coli* S11 is part of the corresponding bacterial S30 subunit. Thus human RPS14 is a ribosomal component fixed early in evolution.

There are many antibiotics and eukaryotic cell poisons that act by inhibiting ribosome function (reviewed in ref. 4). One such drug is emetine, which inhibits protein translation by interacting with the eukaryotic RPS14 subunit to prevent elongation

factor dependent translocation of peptidyl-tRNAs bound to eukaryotic ribosomes in vitro (4).

Chinese hamster ovary (CHO) cell lines resistant to emetine have been shown to contain mutant RPS14 loci (also referred to as the EMTB locus) (5). Such lines have been used to investigate the effects of mutant RPS14 on ribosome function (5-8). Human-CHO cell hybrids are emetine-sensitive, indicating that the EMTB/RPS14 mutation is recessive in CHO cells. This is apparently because arrest of protein synthesis in half of ribosomes blocks translation of all polysomic mRNAs by blocking any functional ribosomes upstream of frozen mutant ribosomes. RPS14 appears to contribute to the structural integrity of the 40S subunit: 40S subunits containing mutant S14 protein are more easily dissociable in high ionic strength wash buffers (9). Ribosomal subunit genes are coordinately expressed in all cells and ribosomal proteins constitute a large fraction of the cell mass in all cell types.

The human RPS14 gene has sequence variances

Rhoads et al. reported the sequence of the human RPS14 gene and cDNA (3). The cDNA contains a 33 nucleotide 5' untranslated region, a 453 nt coding region and a 60 nt 3' untranslated region (including 12 nt of polyA). We undertook a systematic search for DNA sequence variance in the cDNA of RPS14 by analysing 36 unrelated individuals using the single strand conformation polymorphism technique. Primers were designed using the sequence of Rhoads et al. (GENBANK accession M13934, M13641; see ref. 3). SSCP analysis revealed 1 sequence variance, and subsequent DNA sequence analysis confirmed an A *vs.* G transition at nucleotide 183 of the coding sequence. (This change was noted as a difference between the cDNA and genomic sequences in ref. 3.)

As shown in the Target Summary Table, both alleles were detected in all major

populations surveyed, including North American Whites, North American Blacks, Hispanics, Chinese and Japanese.

The human RPS14 gene maps to chromosome 5q23-q33

Dana and Wasmuth (11) used Chinese hamster/human somatic cell hybrids to map the RPS14 gene (designated EMTB) to 5q23-5q35. Later Nakamichi et al. (12) placed the RPS14 gene on the segment 5q23-q33 using similar techniques.

Chromosome band 5q23-q33 is a site of frequent loss of heterozygosity. There have been many studies of LOH on 5q, particularly the 5q21-q22 region where the Adenomatous Polyposis Coli (APC) tumor suppressor gene lies. The most extensively studied cancers are those of the gastrointestinal tract, lung and ovary. The available data on the 5q23-q33 region just distal to APC (where RPS14 lies), suggests that LOH occurs in this region at a frequency of ~30% in cervical cancer (13), 20-40% in colon cancer (14,15), 30-50% in ovarian cancer (16,17), 38% in stomach cancer (18) and 23% in testicular cancer (19). There is also evidence for LOH in head and neck, lung, and liver cancers.

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Example 22: Eukaryotic Initiation Factor 5A (eIF-5A) - Target Gene VARIA351

Initiation Factor 5A is essential for cell growth

Human Initiation Factor 5A (eIF-5A), formerly named Initiation Factor 4D, is an 18-kD protein which promotes formation of the first peptide bond in *in vitro* translation systems - hence the name 'initiation factor' (1,2); however, the full physiological role of eIF-5A is not understood. Inhibition of eIF 5A formation blocks proliferation in all tested cell types (3); the presence of functional eIF 5A has been shown to correlate with the onset of DNA replication (4) - perhaps due to eIF 5A dependent translation of mRNAs encoding proteins necessary for DNA replication (3), and eIF-5A is an essential co-factor for HIV-1 Rev protein (5).

eIF 5A is an unusual protein: one of its lysine residues (amino acid 50) is modified by transfer and hydroxylation of the butylamino-group from the polyamine spermidine to form hypusine, a post translational modification unique to eIF 5A. All of the biological activities of eIF 5A are abrogated in the absence of the hypusine modification, as demonstrated by pharmacological inhibition of hypusine formation in human cell lines (3) and by site directed mutagenesis of the modified lysine residue in the yeast enzyme (6). There are two enzymes responsible for hypusine formation, one of which, deoxyhypusyl hydroxylase, can be inhibited with the drug mimosine (3), providing a convenient pharmacological inhibitor of eFI 5A formation.

The genome of the yeast *Saccharomyces cerevisiae* encodes two eIF 5A genes. Disruption of one (form A) slows growth, disruption of the other (form B) arrests growth and strains with both forms disrupted are non-viable (6). The yeast A form substitutes for human eIF 5A in the mammalian methionyl-puromycin synthesis assay (6), while the human gene complements eIF 5A disrupted yeast (7). eIF 5A is a highly conserved protein, with counterparts in archeae, bacteria and eukaryotes. The yeast proteins are ~63% identical to the human protein (6).

The human eIF 5A gene and mRNA have sequence variances

Smit-McBride, et al. reported the sequence of a human cDNA encoding eIF-5A (8) and Koettnitz et al. (8) later reported the sequence of the active eIF 5A gene, which contains three introns (GenBank accession U17969). A composite sequence made from the cDNA and genomic versions is 1309 nucleotides long and contains a 5' untranslated region of 145 nucleotides, a 462 nt coding region and a 702 nt 3' untranslated region (see annotated sequence). We undertook a systematic search for DNA sequence variance in the cDNA of eIF 5A by analysing 36 unrelated individuals using the single strand conformation polymorphism technique. Primers were designed for amplification. SSCP analysis revealed 2 sequence variances, and subsequent DNA sequence analysis confirmed an A *vs.* G transition at nucleotide 623 and a T *vs.* C transition at nucleotide 1012, both in the 3' untranslated sequence.

Neither sequence variance affects the protein coding sequence, however nucleotide 623 is one nucleotide away from a splice acceptor site at position 622, and could therefore be targeted by an oligonucleotide intended to abrogate splicing in an allele specific manner. The second exonic nucleotide (+2 position) of a splice acceptor site is not highly conserved, nonetheless the A *vs.* G transition at nucleotide 623 may affect the mechanics of splicing.

As shown in the Target Summary Table, both alleles were detected in all major populations surveyed, including North American Whites, North American Blacks, Hispanics, Arabs, Indians and Japanese, except only the nucleotide 1012 variance was detected in the four Chinese surveyed. The overall frequency of heterozygotes was 37% for the nucleotide 623 sequence variance and 52% for the nucleotide 1012 sequence variance.

The human eIF 5A gene maps to chromosome 17p13-p12

Steinkasserer et al. (1995) mapped the eIF 5A gene to 17p13-p12 by fluorescence *in situ* hybridization (9). Three eIF 5A pseudogenes were mapped to 10q23, 17q25 and 19q13.

Chromosome band 17p13-p12 is a site of frequent loss of heterozygosity. There have been many studies of LOH on 17p, particularly the 17p13 region where the p53 tumor suppressor gene maps. Virtually all cancer types have been surveyed for LOH in this area, with particularly extensive studies of breast, colon, ovarian, and stomach cancers. These studies report LOH in approximately 40-60% of breast cancers (10-18), 50-70% of colon cancers (19-25), 25-75% of ovarian cancers (26-30), 20-60% of stomach cancers (31-34), 20-50% of brain cancers (35,36), 45-70% of esophageal cancers (37), 35-65% of non-small cell lung cancers (38,39) and 100% of small cell lung cancers, 15-50% of cervical cancers, 30-80% of head and neck cancers, 20-60% of liver cancers, over 50% of sarcomas and 10-30% of a variety of other cancer types.

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Example 23: Replication Protein A, 32 kDa Subunit (RPA32) - Target Gene VARIA402

The human RPA32 gene encodes a protein essential for cell survival

Replication Protein A (RPA; also known as Replication Factor A, Activator 1, Single Strand Binding Protein or SSB) is a heterotrimeric protein which participates in DNA replication, homologous recombination and nucleotide excision repair (1-3). The evidence that RPA is an essential protein comes from *in vitro* and *in vivo* data.

DNA replication is essential for cell proliferation, as discussed above for RPA70.

The best studied function of RPA32 is in DNA replication. Because of the complexity of DNA replication in higher eukaryotic genomes, the small genome of the papovavirus SV40 has been used as a model system to study DNA replication in human cell extracts. In the 1980s several research groups

developed cell free systems to study DNA replication using SV40 chromosomes as templates (4-8). An effort to identify the minimal set of factors required for DNA replication led to the discovery of RPA. Subsequent work proved that each of the three subunits of RPA is essential for DNA replication (9,10). This was proved in several ways, including by using antibodies to various constituents of the replication complex. Anti-RPA32 antibodies inhibit DNA replication, providing clear *in vitro* evidence for the essential function of this subunit of RPA in human DNA replication (10). The yeast *S. cerevisiae* has a trimeric replication protein A which is structurally and functionally homologous to the human protein. It consists of three subunits similar in size to the human subunits. All three yeast subunits have been disrupted and each disruption produces non-viable yeast (9).

The human RPA32 gene and mRNA are polymorphic.

The published cDNA for the 32 kD subunit of Replication Protein A is 1512 nucleotides long and includes a 5' untranslated segment of 77 nucleotides, followed by a protein coding region of 810 nucleotides and a 3' untranslated region of 625 nucleotides (10). We undertook a systematic search for DNA polymorphism by analysing the RPA32 cDNA from 36 unrelated individuals using the single strand conformation polymorphism technique (described in the methods section). Primers were designed using the sequence of Erdile et al. (GenBank accession J05249; see ref. 10). SSCP analysis revealed 2 variances, one of which was sequenced. Sequencing revealed a G *vs.* A transition at nucleotide 40 of the 5' untranslated region. Four of 36 individuals were heterozygotes, all of them Caucasians. Thus the allele frequency is 25% (4/16) in North American Whites, while no heterozygosity was detected in other populations (see Target Summary sheet).

The RPA32 gene maps to chromosome 1p35

The gene for RPA32 was mapped to chromosome band 1p35 by *in situ* hybridization, somatic cell hybrid analysis and yeast artificial chromosome mapping (11,12). Only one locus was detected by all methods.

Chromosome band 1p35 is a site of frequent loss of heterozygosity. The short arm of chromosome 1 is comparatively well investigated for allele loss, especially in breast and colon cancers. Studies of the 1p35 region show LOH in 15-40% of breast cancers (13,14), ~50% of gliomas (a brain cancer subtype) (15), 20-70% of colon cancers (16,17), ~50% of stomach cancers (18), ~20% of lung cancers (19) and 10-30% of ovarian cancers. High frequency LOH has been detected in several uncommon cancers such as pheochromocytoma (50-80%) and neuroblastoma (~50%).

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Example 24: Replication Protein A, 70 kD subunit (RPA70) - Target Gene VARIA401

The human RPA70 gene encodes a protein essential for cell survival

Replication Protein A (also known as Replication Factor A, Activator or Single Strand Binding protein [SSB]) is a heterotrimeric protein which participates in DNA replication, homologous recombination and nucleotide excision repair (1-3). The evidence that RPA is an essential protein comes from *in vitro, in vivo* and evolutionary data.

DNA replication is essential for cell proliferation, and a variety of antiproliferative drugs act, at least in part, by inhibiting DNA replication. Such drugs include nucleotide analogs that block DNA polymerases, such as 2',3' dideoxy NTPs and 3' deoxy ATP (cordycepin); inhibitors that bind to or modify DNA such as intercalating agents, DNA crosslinking drugs or alkylating agents, and inhibitors that bind to polymerases and replication proteins such as topoisomerase inhibitors like the epipodophyllotoxins, which prevent DNA unwinding necessary for replication (and transcription) and antibiotics which bind to polymerases such as arylhydrazino-pyrimidines.

The best studied function of RPA70 is in DNA replication. Because of the complexity of DNA replication in higher eukaryotic genomes, the small genome of the papovavirus SV40 has been used as a model system to study DNA replication in human cell extracts. In the 1980s several research groups

developed cell free systems to study DNA replication using SV40 chromosomes as templates (4-8). These studies, in seeking to identify the minimal set of factors required for DNA replication, led to the discovery of replication protein A. Subsequent work proved that each of the three subunits of RPA is essential for DNA replications. This was proved in several ways, including by using antibodies to various constituents of the replication complex. These antibodies are effectively inhibitors of RPA70. Anti-RPA70 antibody mediated abrogation of DNA replication provides clear *in vitro* evidence for the essential function of RPA70 in human DNA replication (10). The yeast *S. cerevisiae* has a trimeric replication protein A which is structurally and functionally homologous to the human protein. It consists of three subunits similar in size to the human subunits. The yeast 70 kDa subunit is 31% identical and 75% similar (including conserved amino acids) to its human counterpart (1). All three yeast subunits have been disrupted and each disruption produces non-viable yeast. The yeast 70 kD protein is also a single stranded DNA binding protein.

Single stranded DNA binding proteins (SSBs) are required for DNA replication in a wide variety of organisms, including bacteriophage, bacteria and some DNA viruses of higher eukaryotes. Recently the crystal structure of the DNA binding domain of human RPA was solved and found to be remarkably similar in three dimensional shape to the bacteriophage single stranded DNA binding proteins Pf3 and gene V from f1 phage.

The human RPA70 gene, mRNA and protein have sequence variances

The published cDNA for the 70 kD subunit of Replication Protein A is 2393 nucleotides long and includes a 5' untranslated segment of 69 nucleotides, followed by a protein coding region of 1848 nucleotides and a 3' untranslated region of 476 nucleotides (1). We undertook a systematic search for DNA polymorphism by

analyzing the RPA70 cDNA from 36 unrelated individuals using the single strand conformation polymorphism technique (described in the methods section). Primers were designed using the sequence of Erdile et al. (GenBank accession M63488; see ref. 1). SSCP analysis revealed 5 variances, and subsequent DNA sequence analysis of those variances led to identification of four additional variances. SSCP revealed the variances at nucleotides 81 (G *vs.* A), 1120 (A *vs.* G), 1674 (T *vs.* C), 2050 (T *vs.* C) and 2297, where an insertion/deletion variance of one C nucleotide was observed (8 *vs.* 9 C's in a row). In the course of sequencing around the nucleotide 2297 polymorphism an additional variance was detected at nucleotide 2341 (A *vs.* G). Also, while sequencing additional Swedish individuals around nucleotide 1120 two new variances were observed at nucleotides 1124 and 125 (both C *vs.* T). Finally, in three individuals sequenced for the 2050 variance we noted a difference from the published sequence at nucleotide 2046: we detect 3 T's while the published clone shows just two. This difference may represent another insertion/deletion polymorphism. Five of the nine detected variances are in the coding sequence while four are in the 3' untranslated region.

The frequency of heterozygotes for the five SSCP positive variances ranged from 25-42% among the 36 individuals tested. The small number of individuals genotyped for the other four variances precludes definitive assessment of heterozygosity rates. Some of the polymorphisms appear to occur more commonly in certain racial or ethnic groups (see Target Summary sheet for details). For example, only one of the variances (nt 1674) was detected in Japanese individuals. In general, higher levels of polymorphism were detected in North American Whites than in other groups. The nucleotide 1120 polymorphism, for instance, was heterozygous in 9/36 individuals overall (25%), but in 8/16 North American Whites (50%).

The RPA70 cDNA encodes a 616 amino acid protein. The nucleotide 1120 and 1124 variances result in amino acid substitutions at residues 351 and 352, the former an alanine-threonine exchange (approximately 50% of caucasians are heterozygotes) and

the latter a serine-phenylalanine exchange (rare in the populations tested). In the recently published crystal structure of the DNA binding segment of RPA70 (amino acids 181-422) it is possible to place residue 351 in the second of two tandemly arrayed DNA binding domains (domain B; see ref. 10). Domain B extends from residue I305 to N402, thus the variant residue 351 is in the middle. The published structure is a co-crystal of RPA70 amino acids 181-422 complexed to octadeoxycytosine. Several RPA70 residues contact the oligonucleotide (Figure 4 of ref. 11), including amino acids K343 and T359, which lie 8 residues away from the polymorphism in either direction. Modeling the two variant forms of the protein using the atomic coordinates deposited in the Protein Data Bank (1JMC) should clarify the structural consequences of the alanine-threonine variance. Residue 351 lies in the center of a 50 amino acid segment of the protein that is relatively poorly conserved between yeast and man: 11 of the 50 residues are identical and 25 more are conservative substitutions. Towards the C terminus there is strong conservation: starting 25 residues C-terminal of the polymorphism, 27 of the next 37 residues are identical between yeast and man. Towards the N terminus there is ~30% conservation. Both yeast and human 70 kD RPA subunits contain putative C4-type zinc finger motifs at positions ~480-500.

The RPA70 gene maps to chromosome 17p13.3

The gene for RPA70 has been mapped to chromosome band 17p13.3 by *in situ* hybridization (12). Only one locus was detected.

Chromosome band 17p13.3 is a site of frequent loss of heterozygosity. RPA70 lies just telomeric to the TP53 tumor suppressor gene which is located in cytogenetic band 17p13.1. This region of chromosome 17 is extremely well investigated for allele loss. In general, studies report LOH in approximately 40-60% of breast cancers (13-21), 50-70% of colon cancers (22-28), 25-75% of ovarian cancers (29-33), 20-60% of stomach cancers (34-37), 20-50% of brain cancers (38,39), 45-70% of esophageal cancers (40),

35-65% of non-small cell lung cancers (41,42) and 100% of small cell lung cancers, 15-50% of cervical cancers, 30-80% of head and neck cancers, 20-60% of liver cancers, over 50% of sarcomas and 10-30% of a variety of other cancer types.

Assays developed for RPA: Protein and DNA contacts

Human cDNAs encoding all 3 subunits (70, 34 and 11 kD) of RPA have been cloned and expressed in *E. coli* and in insect cells via baculovirus vectors. The bacterially expressed 70 kDa protein is indistinguishable from its purified human counterpart immunologically and in several functional assays (see Table below). There is good evidence that the 70 kD subunit of RPA interacts with a number of different molecules. A partial list would include the 34 and 11 kD subunits of RPA, DNA, the xeroderma pigmentosum damage recognition and endonuclease proteins XPA and XPG, and DNA polymerase a-primase. These experimentally proven contacts (and almost certainly others) may constrain the topology of the protein in ways that have implications for inhibitor design. In summary a broad array of assays exists to screen for small molecule inhibitors of RPA (possibly including modified nucleotides), that act via competitive, allosteric or protein-protein blocking mechanisms.

Table 4

Assays and reagents available for RPA inhibitor screening

	RPA 70 kD, Assay Systems	
	Purified Human Protein	Purified Bacterial or Baculovirus Protein
ASSAY		
Immunoreactivity	X	X
Single stranded DNA binding	X	X
DNA Polymerase alpha primase	X	X

DNA strand exchange	X	X
Nucleotide excision repair	X	X
Support SV40 Replication	X	X

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Example 25: RNA Polymerase II, 220-kD subunit (RPOL2A) - Target Gene VARIA500

The human RPOL2A gene encodes a protein essential for cell survival

DNA-dependent RNA polymerase II (also known as RPB1 or POLR2A), a complex

multisubunit enzyme, is responsible for the transcription of mRNA from all protein coding genes.

RNA polymerases are found in all cellular organisms. The subunit structure of RNA polymerases is highly conserved in eukaryotes. RNA polymerase acts in concert with as many as 50 other proteins in gene transcription (reviewed in ref. 1). See refs. 2 and 3 for a review of basal transcription by RNA polymerase II and recent progress in identifying and purifying transcription factors and cloning the genes that encode them.

Several subunits of *S. cerevisiae* RPOL2A have been disrupted, always resulting in non-viable yeast.

A variety of inhibitors of RNA polymerase are cytotoxic drugs, such as actinomycin D, which intercalates into double stranded DNA and blocks the movement of RNA polymerase; rifampicin binds the b subunit of *E. coli* RNA polymerase and blocks initiation of transcription. The best studied specific inhibitor of eukaryotic RPOL2A, however, is the potent mushroom toxin - amanitin, a cyclic octapeptide which binds with high affinity (Kd ~10-9 M) to RPOL2A. Several mutations conferring resistance to a-amanitin have been characterized and they all map to the RPOL2A protein coding sequence. Recently a-amanitin binding has been shown to trigger specific degradation of RPOL2A (4).

Damage to actively transcribed DNA is preferentially repaired by the transcription-coupled repair (TCR) system. TCR requires RNA pol II, but the mechanism by which repair enzymes preferentially recognize and repair DNA lesions on PolB II-transcribed genes is incompletely understood.

The human RPOL2A gene and mRNA have sequence variances

Wintzerith et al. and later Mita et al. cloned and sequenced the complete human gene

for RPOL2A (5, 6); the deduced amino acid sequences are identical. The RPOL2A gene contains 29 exons and spans about 32 kb of DNA. The cDNA sequence we evaluated is 6732 nucleotides long (see Annotated RPOL2A Sequence) and contains a 5' untranslated region of 386 nucleotides, a 5910 nucleotide coding region specifying 1970 amino acids, and a 436 nucleotide 3' untranslated region (see annotated sequence). We undertook a systematic search for DNA sequence variance in the cDNA of RPOL2A by analyzing 36 unrelated individuals using the single strand conformation polymorphism technique. Primers were designed for amplification. SSCP analysis revealed 10 sequence variances, and subsequent DNA sequence analysis confirmed a G *vs.* A transition at nucleotide 857, a C *vs.* T transition at nucleotide 1260, a C *vs.* T transition at nucleotide 1346, a C *vs.* T transition at nucleotide 1544, a C *vs.* T transition at nucleotide 1847, a C *vs.* T transition at nucleotide 2678, a C *vs.* T transition at nucleotide 3059, a C *vs.* T transition at nucleotide 3827, a T *vs.* C transition at nucleotide 6466 and a T *vs.* C transition at nucleotide 6557. The former seven sequence variances are in coding sequence and the latter two are in the 3' untranslated sequence. Only one of the ten sequence variances alters the protein coding sequence: the nucleotide 1260 alleles encode arginine (common) or cysteine (rare) at amino acid 292. Only 2/36 individuals surveyed are heterozygotes (6%), however both are North American Whites (2/16 = 12.5%) so further investigation of this population is required. The prevalence of heterozygotes for the other sequence variances varies from 3% to 50%, with 6 sequence variances above 22% (see RPOL2A Target Summary Sheet). The 6 common sequence variances are widely prevalent among all or nearly all the tested populations.

The human RPOL2A gene maps to chromosome 17p13.105

The human RPOL2A gene was initially assigned to the distal portion of the short arm of chromosome 17 (17pter-p12) by *in situ* hybridization and Southern analysis of DNA from human/rodent somatic cell hybrids (7, 8). Subsequent somatic cell hybrid studies narrowed the assignment to 17p13.105-p12 [vanTuinen and Ledbetter (1987)], which

was later confirmed by *in situ* hybridization to 17p13 (9).

Chromosome band 17p13.1 is a site of frequent loss of heterozygosity There have been many studies of LOH on 17p, particularly the 17p13.1 region where the p53 tumor suppressor gene maps. Virtually all cancer types have been surveyed for LOH in this area, with particularly extensive studies of breast, colon, ovarian, and stomach cancers. These studies report LOH in approximately 40-60% of breast cancers (10-18), 50-70% of colon cancers (19-25), 25-75% of ovarian cancers (26-30), 20-60% of stomach cancers (31-34), 20-50% of brain cancers (35,36), 45-70% of esophageal cancers (37), 35-65% of non-small cell lung cancers (38,39) and 100% of small cell lung cancers, 15-50% of cervical cancers, 30-80% of head and neck cancers, 20-60% of liver cancers, over 50% of sarcomas and 10-30% of a variety of other cancer types.

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Example 26: TATA Associated Factor 30 kD subunit (TAF2H) - Target Gene VARIA 520

The human TAF2H gene encodes a component of the transcriptional apparatus

Transcription initiation by RNA polymerase II requires the assembly of a complex of

basic transcription factors which include TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIG/TFIIJ and TFIIH/BTF2 into a preinitiation complex (1,2). TFIID is the first factor to contact the promotor, and subsequent assembly of the transcription complex is dependent on TFIID binding. TFIID is a 700-750 kD multiprotein complex which includes TATA binding protein (TBP) and between eight and 13 TBP-associated factors (TAFs) ranging from 250 to 17 kDa. The TAFs have been shown necessary to reconstitute activation of transcription *in vitro*, leading to the hypothesis that some TAFs link transcription activation domains to the basal transcription complex. The TFIID complex also supports transcription from TATA-less promoters, while TBP fails to do so. Therefore TAFs may also contribute to formation of stable initiation complexes by interacting directly with DNA (2). Conditional temperature sensitive Chinese hamster mutants of another TAF, TAFII250, were detected because, at the non-permissive temperature, DNA synthesis was inhibited leading to arrest of cell division at the G1 phase (3,4). Transfection of a human TAFII250 gene relieved the block at the non-permissive temperature. Thus an essential role has been proven for TAFs in mammalian cells.

A gene (TAF2H) encoding the 30 kDa human TAF protein (TAFII30) was cloned and its functional properties examined by Jacq, et al. (5). The protein was shown to be present in a subset of TFIID complexes and to mediate transcriptional activation by a specific region of the estrogen receptor. Estrogen mediated transcriptional activation could be abrogated by adding an antibody against TAFII30. TAFII30 was not required for basal transcription or for transcription activation by VP-16. It is likely that TAFII30 is required for transcriptional activation by a variety of other transactivating proteins, and is therefore essential for cell proliferation or cell survival.

The human TAF2H gene and mRNA have sequence variants

A human TAF2H cDNA has been cloned and sequenced (5). It encodes a cDNA of 756 nucleotides including a 5' untranslated region of 17 nucleotides, a 657 nucleotide

coding region specifying 218 amino acids, and an 82 nucleotide 3' untranslated region (GenBank accession U13991; see annotated TAF2H cDNA sequence). (Note that the numbering of the sequence in ref. 5 differs slightly from that in the GenBank accession.) We undertook a systematic search for DNA variance in the cDNA of TAF2H by analysing 36 unrelated individuals using the single strand conformation polymorphism technique Primers were designed for amplification. SSCP analysis revealed 1 polymorphism, and subsequent DNA sequence analysis confirmed a G *vs.* A transition at nucleotide 554 (nt 556 of the sequence in ref. 3) of the coding sequence. This variance does not alter the protein coding sequence. Eight of 36 individuals surveyed are heterozygotes (22%). The variance occurs in North American Whites (3/16 = 19%), North American Blacks (2/4) and Hispanics (3/3).

The human TAF2H gene maps to chromosome 11p15.5-p15.2 The human TAF2H cDNA has been mapped to 11p15.5-p15.2 by fluorescent *in situ* hybridization (6). There appears to be a single TAF2H locus. *Chromosome band 11p15-p14 is a site of frequent loss of heterozygosity*

There have been many studies of LOH on 11p, particularly the 11p15 and 11p13 segments where the Beckwith-Weidemann syndrome and WT1 genes reside. As a result there are many studies of LOH in 11p15.5, particularly focusing on breast, cervix, kidney, liver, lung, ovarian, stomach and testicular cancers. These studies show that the 11p15.5 band of chromosome 11 is frequently reduced to one copy (7-24). For example, LOH occurs in approximately 13-33% of breast cancers (7-9), 14-42% of cervical cancers (10), 0-50% of liver cancers (11,12), 0-80% of lung cancers (13-15), 18-54% of ovarian cancers (14,15), 0-71% of stomach cancers (18) and 0-50% of testicular cancers (19,20). Other studies show that 11p15.5 LOH may also be frequent in bladder cancer (21), esophageal cancer (22), some leukemias (23) and sarcomas (24). Many deletions in the 11p15.5 region span relatively short chromosomal segments (2 - 10 megabases; see ref. 13).

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Example 27 - cDNA synthesis

In order to analyze an essential gene for sequence variances, it is generally useful to have a cDNA(s) containing the coding sequence for further sequencing or amplification purposes. cDNAs for some genes are available, however, in some cases it is useful to synthesize the cDNA *de novo*. Methods for obtaining cDNA are known to those skilled in the art, as are methods for sequencing or amplifying the cDNA or portions thereof. An example of a useful cDNA production protocol is provided below, however, as recognized by those skilled in the art, other specific protocols can also be used.

<u>cDNA Production</u>

** Make sure that all tubes and pipette tips are RNase-free. (Bake them overnight at 100oC in the vacuum oven to make them RNase-free.)

1 Add the following to a RNase-free 0.2 ml micro-amp tube and mix gently:

24 ul water (DEPC treated)

12 ul RNA (1ug/ul)

12 ul random hexamers(50 ng/ul)

2 Heat the mixture to 70oC for ten minutes.

3 Incubate on ice for 1 minute.

4 Add the following:

16 ul 5 X Synthesis Buffer

8 ul 0.1 M DTT

4 ul 10 mM dNTP mix (10 mM each dNTP)

4 ul SuperScript RT II enzyme

Pipette gently to mix.

5 Incubate at 42oC for 50 minutes.

6 Heat to 70oC for ten minutes to kill the enzyme, then place it on ice.

7 Add 160 ul of water to the reaction so that the final volume is 240 ul.

8 Use PCR to check the quality of the cDNA. Use primer pairs that will give a ~800 base pair long piece. See "PCR Optimization" for the PCR protocol.

The following chart shows the reagent amounts for a 20 ul reaction, a 80 ul reaction, and a batch of 39 (which makes enough mix for 36) reactions:

	20 ul X 1 tube	**80 ul X 1 tube**	**80ul X 39 tubes**	
water	6 ul	24 ul	936	water
RNA	3 ul	12 ul		RNA
random hexamers	3 ul	12 ul	468	random hexamers
synthesis buffer	4 ul	16 ul	624	synthesis buffer
0.1 M DTT	2 ul	8 ul	312	0.1 M DTT
10mM dNTP	1 ul	4 ul	156	10mM dNTP
SSRT	1 ul	4 ul	156	SSRT

Example 28 - Variance detection by SSCP

This example describes the SSCP technique as used for the identification of sequence variances of the exemplary genes, which were then sequenced to confirm the specific base variances. One common technique currently employed in the identification of such single nucleotide differences is the single strand conformation polymorphism (SSCP) method. (originally described in Orita, *et al.*, "Rapid and Sensitive Detection of Point Mutations and DNA Polymorphisms Using the Polymerase Chain Reaction, *Genomics*, 5:874-879 (1989)) Also employed are restriction fragment length polymorphism (RFLP), heteroduplex analysis, ligase chain reaction (LCR), denaturing gradient gel electrophoresis (DGGE) (Myers, Maniatis, and Lerman, *Methods Enzymol.*, 155:501-527 (1987)) or direct nucleotide sequencing. A review of polymorphism detection techniques, including SSCP, is provided in Grompe, 1993, *Nature Genetics* 5:111-117, which includes a comparison of the commonly used methods.

The SSCP method reveals the presence of sequence variation between individuals as shifts in electrophoretic mobility, but does not show the sequence itself. Direct sequencing of DNAs with altered mobility in the SSCP assay identifies the precise nucleic acid sequence differences among the various alleles. From the nucleic acid sequence data, the amino acid sequence can be determined. One example of the use of this technique is in Pelletier et al., *Cell*, 67:437-447 (1991). The single strand conformation polymorphism methodology is effective for scanning essential genes for sequence variants. It remains the standard technique in human genetics for variance detection, with numerous studies of its efficacy (>90%) and schemes for improved throughput. The SSCP method has been shown to be quite sensitive in the detection of single base changes, for example as shown in Ravnik-Glava et al., 1994, *Human Mol. Genet.* 3:801-807 (human cystic fibrosis gene) and Glava & Dean, 1993, *Human Mutation* 2:404-414 (mouse -globin gene).

A flow chart of the SSCP method as used to identify essential gene sequence variants is shown in Fig. 2 (SSCP OVERVIEW). The method involves the steps of 1) PCR

amplifying a portion of an essential gene cDNA of known sequence (labeled products), 2) selecting restriction enzymes which will produce fragments approximately 100-400 bases in length for 3 independent digestions of the PCR products, 3) heat denaturing the digestion products, 4) running single strand digestion products on non-denaturing gels, 5) identifying bands having different mobilities when compared between individuals, thereby identifying potential sequence variants, 6) sequence at least the region around the potential sequence variance, that region being identified by comparison of the expected fragment sizes resulting from the digestions, 7) record the specific location and base identity of the confirmed sequence variant, 8) calculate the percent occurrence of each sequence variance for the gene as found for the sample of the population. The method is further described in Example 2.

Single strand conformation polymorphism screening is a widely used technique for identifying an discriminating DNA fragments which differ from each other by as little as a single nucleotide. As originally developed by Orita (supra), the technique was used on genomic DNA, however the same group showed that the technique works very well on PCR amplified DNA as well. In the last 8 years the technique has been used in hundreds of published papers, and the modifications of the technique have been described in dozens of papers. The enduring popularity of the technique is due to (1) a high degree of sensitivity to single base differences (>90%) (2) a high degree of selectivity, measured as a low frequency of false positives, and (3) technical ease. SSCP is almost always used together with DNA sequencing because SSCP does not directly provide the sequence basis of differential fragment mobility. The basic steps of the SSCP procedure are described below and summarized in Fig. 2 in flow chart form.

Because the intent of our SSCP screening was to identify as many target gene variances as practically possible, we developed a protocol designed to look at a relatively large number of individuals (36) with a high degree of redundancy, so as to minimize both the false negative and false positive rates.

The 36 individuals examined are reasonably representative of most of the worlds major populations. The racial or geographic origin of the 36 cell lines is detailed in the Target Summary Tables (Figure 5). All cell lines are EBV immortalized lyphoblastoid cells obtained from the Coriell Cell Repository (Camden, NJ), which includes the racial/ethnic/geographic background of cell line donors in its catalog. The cell lines were also selected for their rapid growth rates. In several cases a panel of cDNAs isolated from French Canadians was used instead, or in addition to, the Coriell panel.

SSCP was used to analyze cDNAs (rather than genomic DNAs) because in many cases the full genomic sequence of the target gene is not available, however, the technique is also applicable to genomic sequences. To produce cDNA requires RNA. Therefore each of the 36 cell lines was grown to mass culture and RNA was isolated using the acid/phenol protocol, sold in kit form as TRIAZOL™ by Life Technologies (Gaithersberg, MD). The unfractionated RNA was used to produce cDNA by the action of a modified Maloney Murine Leukemia Virus Reverse Transcriptase, purchased in kit form from Life Technologies (SUPERSCRIPT II™ kit). The reverse transcriptase was primed with random hexamer primers to initiate cDNA synthesis along the whole length of the RNAs. This proved useful later in obtaining good PCR products from the 5' ends of some genes.

Material for SSCP analysis was prepared by PCR amplification of the cDNA in the presence of one ^{32}P labeled dNTP (usually ^{32}P dCTP). Usually the concentration of nonradioactive dCTP was dropped from 200 uM (the standard concentration for all four dNTPs) to about 100 uM, and ^{32}P dCTP was added to a concentration of about 0.1-0.3 uM. This involved adding a 0.3- 1 ul (3-10 uCi) of ^{32}P cCTP to a 10 ul PCR reaction. All radioactivity was purchased from DuPont/New England Nuclear.

The customary practice is to amplify about 200 base pair PCR products for SSCP, however, we found that it was preferable to amplify about 0.8-1.4 kb fragments and

then use several cocktails of restriction endonucleases to digest those into smaller fragments of about 0.1-0.4kb, aiming to have as many fragments as possible between .15 and .3 kb. The digestion strategy had the advantage that less PCR was required, reducing both time and costs. Also, we routinely performed three different digests on each sample (for all 36 cDNAs), and then ran each of the digests separately on SSCP gels. This had the effect of increasing the redundancy of our method, lessening both the false negative and false positive rates. For example: a site of variance might lie within 2 bases of the end of a fragment in one digest, and as a result not affect the conformation of that strand; the same variance, in a second or third digest, would likely lie in a location more prone to affect strand folding, and therefore be detected by SSCP.

After digestion, the radiolabeled PCR products were diluted 1:5 by adding formamide load buffer (80% formamide, 1X SSCP gel buffer) and then denatured by heating to 90%C for 10 minutes, and then allowed to renature by quickly chilling on ice. This procedure (both the dilution and the quick chilling) promotes intra- (rather than inter-) strand association and secondary structure formation. The secondary structure of the single strands influences their mobility on nondenaturing gels, presumably by influencing the number of collisions between the molecule and the gel matrix (i.e., gel sieving). Even single base differences consistently produce changes in intrastrand folding sufficient to register as mobility differences on SSCP.

The single strands were then resolved on two gels, one a 5.5% acrylamide, 0.5X TBE gel, the other an 8% acrylamide, 10% glycerol, 1X TTE gel. The use of two gels provides a greater opportunity to recognize mobility differences. Both glycerol and acrylamide concentration have been shown to influence SSCP performance. The gel apparatus we use (from Owl Scientific, MA) allows 108 samples to be loaded per gel. Since all 36 samples are routinely digested with three different endonuclease mixes there are 108 samples to be analyzed for each PCR product. By routinely analyzing three different digests under two gel conditions (effectively 6 conditions), and by

looking at both strands under all 6 conditions, we achieve a 12-fold sampling of each base pair of cDNA.

All of the sequence variances described in this disclosure were determined by DNA cycle sequencing of ^{32}P labeled PCR products using the femtomole DNA cycle sequencing kit from Promega (WI) and the instructions provided with the kit. Fragments were selected for DNA sequencing based on their behavior in the SSCP assay.

Example 29 - Variance detection by using T4 endonuclease VII mismatch cleavage method

The enzyme T4 endonuclease VII is derived from the bacteriophage T4. T4 endonuclease VII is used by the bacteriophage to cleave branched DNA intermediates which form during replication so the DNA can be processed and packaged. T4 endonuclease can also recognize and cleave heteroduplex DNA containing single base mismatches as well as deletions and insertions. This activity of the T4 endonuclease VII enzyme can be exploited to detect sequence variances present in the general population.

The following are the major steps involved in identifying sequence variations in a candidate gene by T4 endonuclease VII mismatch cleavage:

1. Amplification by the polymerase chain reaction (PCR) of 400-600 bp regions of the candidate gene from a panel of DNA samples The DNA samples can either be cDNA or genomic DNA and will represent some cross section of the world population.
2. Mixing of a fluorescently labeled probe DNA with the sample DNA. Heating

and cooling the mixtures causing heteroduplex formation between the probe DNA and the sample DNA.

3. Addition of T4 endonuclease VII to the heteroduplex DNA samples. T4 endonuclease will recognize and cleave at sequence variance mismatches formed in the heteroduplex DNA.
4. Electrophoresis of the cleaved fragments on an ABI sequencer to determine the site of cleavage.
5. Sequencing of a subset of PCR fragments identified by T4 endonuclease VI to contain variances to establish the specific base variation at that location.

A more detailed description of the procedure is as follows:

A candidate gene sequence is downloaded from an appropriate database. Primers for PCR amplification are designed which will result in the target sequence being divided into amplification products of between 400 and 600 bp. There will be a minimum of a 50 bp of overlap not including the primer sequences between the 5' and 3' ends of adjacent fragments to ensure the detection of variances which are located close to one of the primers.

Optimal PCR conditions for each of the primer pairs is determined experimentally. Parameters including but not limited to annealing temperature, pH, $MgCl_2$ concentration, and KCl concentration will be varied until conditions for optimal PCR amplification are established. The PCR conditions derived for each primer pair is then used to amplify a panel of DNA samples (cDNA or genomic DNA) which is chosen to best represent the various ethnic backgrounds of the world population or some designated subset of that population.

One of the DNA samples is chosen to be used as a probe. The same PCR conditions used to amplify the panel are used to amplify the probe DNA. However, a

flourescently labeled nucleotide is included in the deoxy-nucleotide mix so that a percentage of the incorporated nucleotides will be fluorescently labeled.

The labeled probe is mixed with the corresponding PCR products from each of the DNA samples and then heated and cooled rapidly. This allows the formation of heteroduplexes between the probe and the PCR fragments from each of the DNA samples. T4 endonuclease VII is added directly to these reactions and allowed to incubate for 30 min. at 37 C. 10 ul of the Formamide loading buffer is added directly to each of the samples and then denatured by heating and cooling. A portion of each of these samples is electrophoresed on an ABI 377 sequencer. If there is a sequence variance between the probe DNA and the sample DNA a mismatch will be present in the heteroduplex fragment formed. The enzyme T4 endonuclease VII will recognize the mismatch and cleave at the site of the mismatch. This will result in the appearance of two peaks corresponding to the two cleavage products when run on the ABI 377 sequencer.

Fragments identified as containing sequencing variances are subsequently sequenced using conventional methods to establish the exact location and sequence variance.

Example 30 - Identification of Sequence Variances by Informatics-based analysis of gene-sequence databases

In addition to and/or in conjunction with the molecular biology based approaches for identifying sequence variances in genes, particularly in essential genes, such sequence variances can be identified by analysis of public and/or private genetic sequence databases. Such information can be either genomic or cDNA sequence information.

The data base analysis process includes the following major steps:

1.

1. capture of homologous sequences of a particular gene from data bases. It is preferable to obtain a large number of independent sequences of a particular gene

2. analysis of collected sequences of a particular gene to identify authentic sequence variances. This step involves the discrimination of authentic sequence variances, which are sequence variances which actually exist in the population, from sequencing errors and artifacts. It is expected that about 0.1-0.3% of the bases will occur as true variances, while the frequency of sequencing artifacts is expected to be 1-3%. This discrimination utilizes the expected frequencies of occurrence of specific types of nucleotide sequence changes. Such information includes the characteristic frequency of specific transitions and transversions and of the characteristic frequency of deletions and insertions in authentic variations. It uses the frequency of occurrence of known types of sequencing artifacts such as single base insertions or deletions adjacent to repeated C or G nucleotides. Additional information for such discrimination is provided if particular putative authentic variations are observed in multiple independently derived sequences of the gene.

An implementation of this sequence variance identification process utilizes a reference sequence of an essential gene. Preferably, the reference sequence is a high quality sequence, meaning that there is a low frequency of occurrence of sequencing errors or artifacts. The second step is the retrieval of allelic sequences of that essential gene from available databases such as the BLAST server, the UNIGENE database, or other such sequence database. Such allelic sequences need not be complete, but are preferably long enough to ensure that they are in fact allelic sequences. The third step involves alignment analysis to identify and tabulate sequence differences between the different available sequences. An algorithm for such analysis is the Smith-Waterman local alignment algorithm. Use of an algorithm of this type involves a series of pair-

wise alignments of each retrieved sequence with the reference sequence. The fourth step involves analysis of the observed sequence differences and assignment of a probability that each sequence difference represents an authentic variance. This analysis utilizes program filters which are combined in a weighted fashion to determine a final probability . Such program filters include comparison of the observed difference with common mutational changes and sequencing errors, a weighting of the reliability of a particular retrieved sequence based on the total number of differences observed, a weighting based on the location within a retrieved sequence where a change was observed and a significant weighting based on the observance of a particular difference in multiple independently derived retrieved sequences.

Using such an implementation, a database analysis with respect to a particular reference sequence produces a list of putative authentic sequence variances and a probability for each of those variances that the sequence difference is an authentic variance. As described above, the probability is obtained through the use of a series of weighted program filters and thus these filters are modified to produce optimal authentic variance discrimination.

Example 31 - Antiproliferative effects of variance specific inhibition of RPA70

This example describes experiments showing the practicality and utility of variance-specific inhibition of essential genes for cancer therapy. Specifically, this example describes i*n vitro* experiments showing the design and production of variance-specific oligonucleotides for antisense inhibition of variant alleles of the essential Replication Protein A, 70 kDa subunit (RPA70) for inhibition of RPA70 mRNA, and the use of these oligonucleotides to inhibit cell proliferation and to reduce the number of cells in a variance-specific manner.

Variance-specific inhibition and cell killing with antisense oligonucleotides against

RPA70

These experiments with RPA70 illustrate the feasibility of each of the steps for development of a variance specific inhibitor:

Select candidate target gene essential for cell survival or proliferation. As described above, RPA is essential for replication in prokaryotic and eukaryotic cells, mitochondria, phage, viruses and in *in vitro* (SV40) replication systems. The protein is a heterotrimer required for loading DNA polymerase onto the DNA template during cell replication. The 70 kDa subunit, RPA70, is a single strand binding protein that mediates the interaction of RPA with DNA. Without this protein, the replication complex does not associate with DNA and the replication of DNA does not occur.

Confirm chromosome location and LOH frequency. RPA70 is encoded by a single gene locus on chromosome 17pl3.3, immediately adjacent to the p53 gene at 17p13.1. LOH involving chromosome band 17pl3.3 has been documented in 50-70% of colon, lung, breast, and ovarian cancers. LOH at this locus also occurs in other cancers. The inventor as confirmed LOH involving RPA 70 in breast, colon, lung and other cancers.

Identify common variances in the normal population. We have identified five common variances in the RPA70 gene (Figure 8). The most common occurs in 42% of the normal population. One variance alters the amino acid sequence and is present in 25% of the normal population (44% of Caucasians). This variance occurs within the active DNA binding domain (discussed below). These variances are described in the description above and in Fig. 1.

Demonstrate antiproliferative effects due to inhibition of candidate gene. The inventor has shown that inhibition of RPA70 in T24 bladder carcinoma cells with an antisense oligonucleotide reduces cell number. This effect is comparable to treatment of these cells with antisense oligonucleotide against *ras*, previously shown to have antitumor

effects *in vitro* and *in vivo* (Figure 9).

Design variance-specific inhibitor. Variance specific antisense oligonucleotides were designed to differentially inhibit the two variant forms of RPA70. Experiments were performed using tumor cell lines that are homozygous for each form of the target gene. Figure 10 shows inhibition of mRNA levels in Mia Paca II cells by the 13085 oligonucleotide which matches the variance in these cells. In contrast, in T24 cells (and A549 cells, see below) the 12781 oligonucleotide matches the target gene and inhibits mRNA levels. In both cell lines neither the control oligonucleotide differing by one base (13085 in T24 cells and 12781 in Mia Paca II cells) nor a random-sequence oligonucleotide control (13706) inhibit mRNA levels to the same extent as the matched oligonucleotide.

Figure 10 demonstrates that the RPA 70 mRNA can be specifically down regulated in an allele-specific manner. However, the 13085 oligomer used also has a small effect on the level of the unmatched RNA. In order to increase the discrimination we altered the structure of the targeting oligomer, 13085. The results are shown in Figure 11. By shortening the oligomer we retain its ability to down-regulate its matched target RNA (Mia Paca II cells, right half of Figure 11). Strikingly, however, this alteration dramatically altered the ability of this oligomer to down-regulate the mismatched variant RNA T24 cells, left half of Figure 11. The reciprocal regulation by oligomer 12781 was augmented by altering transfection conditions. These data suggest that even simple changes to the rudimentary "first generation" chemistry and transfection techniques can have significant effects in enhancing the ability of the oligomers to recognize and down regulate specific mRNAs.

Achieve variance-specific antiproliferative effects in cancer cells. Cell proliferation in each cell line, determined by BrdU incorporation, was suppressed to a greater degree by the matched oligonucleotide than by the controls differing by one base (Figure 12).

Cell proliferation in A549 cells was inhibited by oligomer 12781 to a greater degree than by oligomer 13085. Cell proliferation in Mia Paca 11 cells was inhibited more by oligomer 13085.

Additional studies were performed to characterize the antiproliferative effect in A549 cells (12781 genotype). A dose response curve demonstrates inhibition of BrdU incorporation by the matched oligonucleotide (12781) at concentrations 8-fold lower than the oligonucleotide with one base mismatch (13085) (Figure 13).

Cell survival was measured by staining cells with Sulforhodamine B dye 72 hours after treatment with oligonucleotides. Dose dependent reductions in cell number were observed in cells treated with the matched oligonucleotide (12781) but not with an oligonucleotide containing the one base mismatch (13085) (Figure 14). In contrast, in Mia Paca II cells, more cell killing was observed with the 13085 oligonucleotide than with the 12781 oligonucleotide (Figure 15). The oligonucleotides used in these studies have not been optimized for achieving allele-specific effects. Oligonucleotides using advanced chemistries can be utilized to optimize the potency and provide greater discrimination between variant targets at lower levels.

Example 32 - variance specific inhibition of essential genes

This example describes experiments showing the practicality and utility of variance-specific inhibition of essential genes for cancer therapy including RNA Pol II, and ribonucleotide reductase. Specifically, this example describes i*n vitro* experiments showing the design and production of variance-specific oligonucleotides for antisense inhibition of variant alleles of the essential Ribonuclotide Reductase (RR), the design and production of variance-specific oligonucleotides against RR, and the use of these oligonucleotides to inhibit RR mRNA in a variance-specific manner.

Variance-specific inhibition of Ribonucleotide Reductase.

Ribonucleotide Reductase (RR) is an essential gene of nucleoside metabolism. Inhibitors of this function are known to be cell lethal. Two variances were discovered at position 2410 and 2419. Oligonucleotides were synthesized to a sequence spanning these two variations. In one case the oligomer targeted the GnnnnnnnnA variation (oligomer Varia 2410GA or RR2410GA) and in the other case the oligomer targeted the AnnnnnnnnG variant (oligomer Varia 2410AG or RR2410AG). In Mia Paca II cells which contain the GnnnnnnnnA variance, the RR2410GA antisense oligomer dramatically knocked down the level of RR mRNA. However, the oligomer targeting the other variance, oligomer Varia 2410AG, had little to no effect on the level of mRNA (Figure 16). The reciprocal regulation was demonstrated in MDA-MB 468 cells which express the other variance, AnnnnnnnnG (Figure 17). In these cells Varia 2410AG dramatically lowered the level of RR mRNA. In contrast, Varia 2410GA had no effect on the level of mRNA. These data taken together, are another example of allele-specific targeting of gene expression. We are also determining the effect of down regulating RR gene expression on cellular growth.

Example 33 - variance specific inhibition of essential genes using advanced oligonucleotide chemistries.

This example describes experiments showing the practicality and utility of variance-specific inhibition of essential genes for cancer therapy. Specifically, this example describes i*n vitro* experiments showing the design and production of variance-specific oligonucleotides for antisense inhibition of variant alleles of the essential Glutamyl/prolyl tRNA Synthetase (EPRS), the design and production of variance-specific oligonucleotides against EPRS, and the use of these oligonucleotides to inhibit EPRS mRNA in a variance-specific manner.

Glutamyl-prolyl-tRNA synthetase (EPRS) is an essential gene, required for the synthesis of both glutamic acid tRNA and proline tRNA. Without EPRS protein synthesis is blocked. Two variances were discovered in this gene at positions 2963 and 2969 in the cDNA. We have demonstrated variance-specific inhibition of this gene with antisense oligonucleotides exploiting several different types of chemistry.

The experiments described above with RPA70 and RR utilized phophorothioate chemistry. This chemistry was developed to achieve greater stability *in vivo,* and this compound ha been used in several successful clinical trials. Phosphorothioates, however have low affinity for the RNA target, and, consequently, relatively lower specificity. We have achieved improved variance-specific inhibition using alternative chemistries. Specifically, we have synthesized hybrid oligonucleotides that contain both phosphorothioate and nucleotides with higher affinities. These hybrids contain "wings" consisting of six nucleotides with a 2' sugar modification (ethoxy-methoxy radical at the 2' position) and either a phosphorothioate or phosphodiester backbone. Between the "wings" is a 8 nucleotide sequence of phosphorothioates that overlaps the variance. (In these constructs the 5' position of cytosine has been methylated.) As shown in Figure 18, variance specific inhibition is observed with the conventional phorphorothioates. Greater inhibition of target mRNA is observed using the hybrid chemistries at lower doses. Inhibition by the matched hybrid oligomer, 14977, occurs at approximately 50-100 nM. The effect is extremely oligomer-specific. The mismatched oligomer, 14971, has no effect on mRNA levels at concentrations as high as 400 nM (Figure 19).

Example 34 - *in vivo* cancer therapy using oligonucleotides

This example describes reported *in vitro and in vivo* data on the treatment of cancer in animal models using antisense oligonucleotides against c-raf, showing the expected

correlation between *in vitro* suppression of mRNA and cell proliferation with oligonucleotides, and *in vivo* anticancer activity.

In vitro evidence for inhibition of mRNA by antisense oligonucleotides and inhibition of cell proliferation is commonly used to predict *in vivo* effects on tumors. This is exemplified by the publication by Monia et al (Nature Medicine, Volume 2 Number 6, June 1996) who demonstrated anticancer effects using oligonucleotides against C-raf kinase. *In vitro* treatment of human tumor cells with appropriate phosphorothioate antisense oligomers led to specific inhibition of C-raf kinase gene expression and subsequent decrease in cellular proliferation, IC50=50-100nM. Administration of C-raf antisense oligomers to nude mice having a tumor burden derived from these cells significantly inhibited tumor growth *in vivo,* IC50= 0.06-0.6 mg/kg. Remarkably, the investigators were able to show that the anti-C-raf oligomers down-regulated the level of C-raf kinase mRNA *in vivo* by assaying mRNA levels in cells removed from the tumor.

Example 35 - in vivo cancer therapy by oligonucleotide inhibition of ras

This example describes reported i*n vivo* data showing an anticancer effect using an allele-specific inhibitor for suppression of mutant H-ras. Schwab *et al (Proc. Nat. Acad. Sci. USA* 91:10460-464, Oct 1994) demonstrated antitumor effects of an antisense oligonucleotide specific for the mutant ras in animal models. In these experiments HBLl00 cells were transformed with the RAS oncogene. *In vitro* studies demonstrated that the RAS mRNA could be specifically down-regulated by a nanoparticle conjugated phosphodiester antisense oligomer. Only the transforming RAS mRNA was targeted by the oligomer. The normal cellular RAS mRNA, differing by a single base, was not affected by the antisense oligomer. The decrease in RAS expression was associated with a decrease in the growth rate of the cells. The

transformed HBL100 cells were injected into nude mice to form tumors; following subcutaneous injection of nanoparticle-conjugated phosphodiester antisense oligomers, Schwab et al measured both a decrease in targeted tumor weight and volume. Specificity for tumor cell growth correlated well with the *in vitro* data having a 5-fold differential between antisense and control groups.

The authors of this paper are proceeding with clinical trial of these oligonucleotides for the treatment of cancer, demonstrating the potential clinical utility of these methods.

Example 36. Variance detection by DGGE

This example describes denaturing gradient gel electrophoresis (DGGE), a technique used for the identification of DNA sequence variances in genomic DNA, cDNA or in PCR products amplified from genomic DNA or cDNA. The DGGE method was originally described by Fischer and Lerman (Two Dimensional Electrophoretic Separation of Restriction Enzyme Fragments of DNA. Methods in Enzymology, vol. 68: 183-191, 1979; DNA Fragments Differing by Single Base-Pair Substitutions are Separated in Denaturing Gradient Gels: Correspondence with Melting Theory. Proc. Natl. Acad. Sci. U.S.A. 80:1579, 1983) and has been improved since then by many investigators. See, for example: Myers, et al., Mutation Detection by PCR, GC-Clamps, and Denaturing Gradient Gel Electrophoresis, pp. 71-88 in Erlich, H.A., editor: PCR Technology: Principles and Applications for DNA Amplification, Stockton Press, New York, 1989; Myers, et al., Detecting Changes in DNA: Ribonuclease Cleavage and Denaturing Gradient Gel Electrophoresis, *in* Davies, K.E., editor: Genomic Analysis: A Practical Approach, IRL Press Ltd., Oxford, 1988, pp. 95-139; E.S. Abrams and V.P. Stanton Jr., Use of Denaturing Gradient Gel Electrophoresis, pp. 71-104 *in* Lilley, D.M.J. and Dahlberg, J.E., editors: DNA Structures, Part B: Chemical and Electrophoretic Analysis of DNA, Methods in

Enzymology, volume 212, Academic Press, 1992; .) Descriptions of current applications of the technique can be found in

The basic principal of DGGE involves the creation of a gradient of denaturant in a gel, which is then used to resolve double stranded DNA (or RNA) fragments on the basis of conformational differences associated with strand melting. The denaturant can be chemical (as in DGGE, where a gradient of formamide and urea is typically used) or thermal (as in a related technique called thermal gradient gel electrophoresis, or TGGE, where a gradient of heat is used). To obtain conditions where double stranded DNA is close to melting, DGGE gels are immersed in a heated bath of electrophoresis buffer, while TGGE gels have a fixed concentration of chemical denaturant.

As a double stranded DNA molecule migrates through a DGGE gel from a low concetration of denaturant at the origin to higher concentrations of denaturant toward the end of the gel it eventually reaches a level of denaturant that will cause partial melting. (Some design of DNA molecules is often necessary to assure that the partial melting will occur as desired; see below.) The concentration of denaturant required to melt a given DNA segment is highly sensitive to sequence differences in the DNA, including changes as subtle as a single nucleotide substitution. Partially melted DNA fragments move through gels at a much slower rates than their fully duplex counterparts. Thus two DNA fragments differing at a single nucleotide can be distinguished on the basis of their gel position after an appropriate period of electrophoresis: the fragment with the more stable structure (resulting from, for example, a G:C base pair in place of an A:T pair) will travel further in the gel than its less stable counterpart, because it will encounter the concentration of gradient required to melt it (and consequently dramatically retard or nearly stop its movement) at a point further along in the gel.

The DGGE method reveals the presence of sequence variation between individuals as

shifts in electrophoretic mobility, but does not show the sequence itself. Direct sequencing of DNA fragments (from different individuals) with altered mobility in the DGGE assay will reveal the precise sequence differences among them (see example 37, Variance Detection by DNA Sequencing). From the nucleic acid sequence data, the amino acid sequence can be determined and any amino acid differences can be identified.

The DGGE method is suitable for analysis of restriction enzyme digested genomic DNAs, as initially described by Lerman and co-workers (*supra*) and later extended (Gray, M. Detection of DNA Sequence Polymorphisms in Human Genomic DNA by Denaturing Gradient Blots, American Journal of Human Genetics, 50: 331-346, 1992). DGGE is equally suitable for analysis of cloned DNA fragments or DNA fragments produced by PCR. The analysis of cloned fragments or PCR fragments has the advantage that non-natural sequences, rich in G and C nucleotides can easily be added to the 5' ends (either flanking the cloning site or at the 5' ends of PCR primers). Such DNA fragments have very stable double stranded segments, called GC clamps, at one or both ends. The GC clamps alter the melting properties of the fragments, and can be designed so as to insure melting of the inter-primer segment of the PCR product at a lower temperature than the clamps, thereby optimizing the detection of sequence differences (see Myers *et alia*, *supra* and Myers *et alia*, Nearly All Single Base Substitutions in DNA Fragments Joined to a GC Clamp Can be Detected by Denaturing Gradient Gel Electrophoresis. Nucleic Acids Research 13: 3131, 1985). GC clamps can be rationally designed for any specific DNA fragment of known sequence by use of a computer program (MELT87, written by L. Lerman) that accurately predicts melting behavior based on analysis of primary sequence. When GC clamps are used correctly, the DGGE method is highly efficient at detecting DNA sequence differences. Not only are nearly 100% of differences detected, but the false positive rate is essentially zero. (Abrams, E.S., et alia, Comprehensive Detection of Single Base Changes in Human Genomic DNA Using Denaturing Gradient Gel

Electrophoresis and a GC Clamp. Genomics 7: 463-475, 1990.) Recently methods for increasing the throughput of DGGE have been developed, based on multiplex PCR.

The steps in carrying out DGGE with GC clamps are:

1. *Design DNA fragments with optimal melting behavior.* Select oligonucleotide primers, using GC clamps as necessary, to produce a single melting domain over the length of the sequence to be analyzed. (It may be necessary to divide the sequence into overlapping fragments to achieve this goal.) Design of primers and simulated analysis of fragments can be performed with the computer program described by Lerman. (Lerman, L.S. and Silverstein, K. Computational Simulation of DNA Melting and its Application to Denaturing Gradient Gel Electrophoresis. Methods in Enzymology 155: 482-501, 1987.) The output of the program is the melting map of the fragment, from which it will also be possible to determine the optimal range of denaturant in the gradient and the approximate electrophoresis time for fragments to reach the point of melting in the gradient.

2. *Amplify the fragment by PCR.* Procedures for optimizing PCR are briefly described in other examples and are well known in the art. Template DNA samples can either be cDNA or genomic DNA and will typically be drawn from a panel of unrelated individuals.

3. *Pour a denaturing gradient gel.* Briefly, make up two gel solutions containing the desired beginning and end concentrations of denaturant. The gel solutions are generally made up by mixing "0%" and "100%" denaturant stock solutions, where the 0% stock consists of 7% acrylamide in Tris-acetate EDTA (TAE) electrophoresis buffer, and the 100% stock is also 7% acrylamide in TAE, plus 40% formamide by volume and 7 molar urea. Equal volumes of the two solutions (e.g. twelve milliliters of each solution) are poured into the two chambers of a gradient maker (usually between 20 and 40% denaturant in the upstream chamber and 60 to 80% in the lower

one) immediately after addition of ammonium persulfate and TEMED for acrylamide polymerization. Open the stopcock of the gradient maker and pour the gradient gel. Usually gels are .75 to 1 mm in thickness, and gel combs that form 10-30 wells are used. With commercially available apparatus multiple gradient gels can be poured simultaneously. Suitable apparatus is sold by several vendors, including the BioRad (Hercules, CA) Dcode system and the C.B.S. Scientific DGGE system.

4. *Place the gel in a heated bath of electrophoresis buffer.* Gels are electrophoresed at elevated temperature which, together with the denaturant, brings the DNA fragments to their melting point. Gels are often run at 60°C in 1X TAE buffer, with constant recirculation of buffer to the upper buffer chamber. Once the gel has been placed in the heated tank and allowed to equilibrate it can be loaded. Multiple gels can be run simultaneously in the same tank with the apparatus listed above.

5. *Load and run gel.* Usually enough PCR product from each sample is loaded on the gel so that samples can be detected by a simple DNA staining procedure; use of radioactivity, dyes or hybridization procedures can thereby be avoided. At least 100 mg of each sample should be loaded, but preferably over 200 ng. Gel running conditions can be estimated from the output of the MELT87 program, however empirical adjustment will often be necessary. Usually a voltage of ~80 to 200V is applied for periods of 5-20 hours, depending on the characteristics of the fragments being analyzed.

6. *Stain and analyze gel.* After electrophoresis gels are stained with ethidium bromide, SYBR Green, silver or some other procedure. The location of PCR products produced with the same primer pairs should be compared. Altered location, and usually the appearance of two or more bands instead of one, signify the presence of DNA sequence differences. (The reason for more than two bands from a diploid sample is that during the terminal cycle of heating and cooling of the PCR

step heteroduplexes are formed between the maternally and paternally inherited alleles. If those alleles differ in sequence, the heteroduplexes will have mispaired nucleotides at the sites of difference. As a result the heteroduplexes will be less stable than either of the homoduplex species, and will consequently melt and be retarded in the gel at a lower concentration of denaturant. Altogether one may see four bands in such samples: two reciprocol heteroduplexes and two homoduplexes.) The specific pattern of fragments in each lane constitutes a signature for a specific nucleotide change.

7. *Sequence DNA fragments with altered mobility*. Examples of all different signatures should next be analyzed by DNA sequencing to identify the base difference(s) accounting for altered mobility in the gradient gel. See example 37 for a description of this procedure and the subsequent steps of recording the sequence variances and analyzing their frequency and structural and functional consequences.

Example 37: Variance detection by sequencing.

Sequencing by the Sanger dideoxy method or the Maxim Gilbert chemical cleavage method is widely used to determine the nucleotide sequence of genes. Presently, a worldwide effort is being put forward to sequence the entire human genome. The Human Genome Project as it is called has already resulted in the identification and sequencing of many new human genes. Sequencing can not only be used to identify new genes, but can also be used to identify variations between individuals in the sequence of those genes.

The following are the major steps involved in identifying sequence variations in a candidate gene by sequencing:

1. Amplification by the polymerase chain reaction (PCR) of 400-700 bp regions of the candidate gene from a panel of DNA samples The DNA samples can either be cDNA or genomic DNA and will represent some cross section of the world population.
2. Sequencing of the resulting PCR fragments using the Sanger dideoxy method. Sequencing reactions are performed using flourescently labeled dideoxy terminators and electrophoresedon an ABI 377 sequencer or its equivalent.
3. Analysis of the resulting data from the ABI 377 sequencer using software programs designed to identify sequence variations between the different samples analyzed.

A more detailed description of the procedure is as follows:

A candidate gene sequence is downloaded from an appropriate database. Primers for PCR amplification are designed which will result in the target sequence being divided into amplification products of between 400 and 700 bp. There will be a minimum of a 50 bp of overlap not including the primer sequences between the 5' and 3' ends of adjacent fragments to ensure the detection of variances which are located close to one of the primers.

Optimal PCR conditions for each of the primer pairs is determined experimentally. Parameters including but not limited to annealing temperature, pH, $MgCl_2$ concentration, and KCl concentration will be varied until conditions for optimal PCR amplification are established. The PCR conditions derived for each primer pair is then used to amplify a panel of DNA samples (cDNA or genomic DNA) which is chosen to best represent the various ethnic backgrounds of the world population or some designated subset of that population.

PCR reactions are purified using the QIAquick 8 PCR purification kit (Qiagen cat#

28142) to remove nucleotides, proteins and buffers. The PCR reactions are mixed with 5 volumes of Buffer PB and applied to the wells of the QIAquick strips. The liquid is pulled through the strips by applying a vacuum. The wells are then washed two times with 1 ml of buffer PE and allowed to dry for 5 minutes under vacuum. The PCR products are eluted from the strips using 60 ul of elution buffer.

The purified PCR fragments are sequenced in both directions using the Perkin Elmer ABI Prism™ Big Dye™ terminator Cycle Sequencing Ready Reaction Kit (Cat# 4303150). The following sequencing reaction is set up: 8.0 ul Terminator Ready Reaction Mix, 6.0 ul of purified PCR fragment, 20 picomoles of primer, deionized water to 20 ul. The reactions are run through the following cycles 25 times: 96°C for 10 second, annealing temperature for that particular PCR product for 5 seconds, 60°C for 4 minutes.

The above sequencing reactions are ethanol precipitated directly in the PCR plate, washed with 70% ethanol, and brought up in a volume of 6 ul of formamide dye. The reactions are heated to 90°C for 2 minutes and then quickly cooled to 4°C. 1 ul of each sequencing reaction is then loaded and run on an ABI 377 sequencer.

The output for the ABI sequencer appears as a series of peaks where each of the different nucleotides, A, C, G, and T appear as a different color. The nucleotide at each position in the sequence is determined by the most prominent peak at each location. Comparison of each of the sequencing outputs for each sample can be examined using software programs to determine the presence of a variance in the sequence. One example of heterozygote detection using sequencing with dye labeled terminators is described in Pui-Yan Kwok *et. al.* (Pui-Yan Kwok, Christopher Carlson, Thomas D. Yager, Wendy Ankener, and Deborah A. Nickerson, *Genomics* 23, 138-144 (1994)). The software compares each of the normalized peaks between all the samples base by base and looks for a 40% decrease in peak height and the concomitant

appearance of a new peak underneath. Possible variances flagged by the software are further analyzed visually to confirm their validity

Example 38. Loss of heterozygosity.

Loss of chromosomes or segments of chromosomes in disease cells results in loss of alleles in the disease cells compared to normal diploid cells. Such allele losses are a common occurrence in cancer, where they have been documented in over 1,500 publications in the past 14 years. More recent work has documented the occurrence of allele loss in other proliferative diseases. Several cytogenetic and molecular techniques have been developed to measure chromosome losses. The molecular techniques are preferable for identification of allele loss because they also show which allele is lost, and are therefore best suited to provide the information needed to implement the present invention.

In order to measure chromosome loss using molecular techniques it is necessary to be able to distinguish the paternally and maternally inherited copies of a given chromosome. DNA variances allow the two copies of a given chromosome to be distinguished because different alleles can be resolved electrophoretically. The standard method for analyzing allele loss in cancer is to compare tumor cell DNA with normal cell DNA, either in a Southern blot or using PCR based techniques. A patient's tumor DNA is said to be "informative" for allele loss only at loci where the patient's normal cells are heterozygous. When such heterozygous loci are examined in tumor cells often only one allele is detected. Such tumor cells have lost the heterozygous state which characterizes all normal somatic cells of the patient, hence the term loss of heterozygosity (LOH).

Several effective molecular procedures have been developed to measure LOH. These procedures have been applied most extensively to cancer tissues, however the same methods are effective in the study of nonmalignant diseases such as atherosclerotic plaques and endometriosis. The main steps are:

1. *Identify DNA variances at or near the locus to be investigated for LOH.*

LOH usually affects large segments of DNA, ranging from several megabases to an entire chromosome. As a result, accurate estimation of LOH at a specific locus can be obtained by measuring the frequency of LOH at neighboring polymorphic markers on the same chromosome, or more preferably on the same chromosome arm, or most preferably within several 10-20 megabases of the locus. However, to precisely measure LOH at a specific locus requires a variance at the locus. Different types of variances have been used to study LOH, including single nucleotide polymophisms (SNPs), specifically SNPs that alter restriction endonuclease cleavage sites, called RFLPs. (For details of this approach see Vogelstein, B., et al., Allelotype of colorectal carcinomas. *Science* 244: 207-211, 1989). Also short tandem repeat polymorphisms (STRPs), including di-, tri- and tetranucleotide repeat polymorphisms have been used to measure LOH. (For details of this procedure see Jones and Nakamura, Deletion Mapping of Chromosome 3p in Female Genital Tract Malignancies Using Microsatellite Polymorphisms. Oncogene 7: 1631-1634, 1992.) Procedures for identifying variances are described in Examples 28, 29, 30 and 36.

2. *Prepare DNA from paired normal and disease tissue samples from patients being studied.*

Before preparing genomic DNA from tumor tissue it is important to assess tumor cell purity and viability, using microscopic examination of frozen sections if necessary. If embedded pathological specimens are being analyzed tumor cell purity can be

assessed by examining histologic sections before selecting areas for cell isolation and DNA purification. (See Johnson, et al., Direct Molecular Analysis of Archival Tumor Tissue for Loss of Heterozygosity, BioTechniques 19:190-191, 1995, and references therein for description of techniques for purifying tumor cell DNA from archival pathology samples.) Areas of necrosis and extensive admixture of normal and tumor tissue should be avoided. For Southern blotting ~5-10 ug of genomic DNA is required for each sample being analyzed. For PCR based methods as little as 5 to 10 ng of genomic DNA is sufficient; much less will suffice if two successive rounds of PCR amplification are used.

3. *Determine genotype in the normal and disease tissues using a quantitative or semi-quantitative procedure that allows the amount of each allele to be measured. Compare the ratio of alleles in the normal tissue to the ratio in the tumor tissue*

In order to show LOH at a given locus it is necessary to establish that the patient is constitutionally heterozygous at the locus. Thus DNA from normal tissue must be tested, either before or in parallel with tumor tissue DNA. A variety of methods can be used for quantitation of signal from the two alleles. If the alleles are compared on a Southern blot then signal in the bands corresponding to the two alleles can be counted by radioactive or nonradioactive techniques (see Ausubel, et al., Current Protocols in Molecular Biology, John Wiley & Sons). One method employs phosphor technology using a Molecular Dynamics PhosphorImager with ImageQuant software to measure signals. If the alleles are compared after PCR amplification then DNA sequencing can provide accurate quantitation of allele ratios. See, for example, Goldsborough and Kornberg, Allele-Specific Quantification of Drosophila Engrailed and Invected Transcripts, Proc. Natl. Acad. Sci. U.S.A. 91:12696-12700, 1994.

Using highly variable markers distributed across the genome a comprehensive map of LOH can be assembled for a specific cancer type. Such data sets have been termed allelotypes. Separate studies are necessary for different cancer (or other disease) types

as the patterns of LOH differ significantly in different diseases.

Other techniques that have been used to detect allele loss in cancer include Comparative Genomic Hybridization (CGH) and Representation Difference Analysis (RDA) however these methods are more complex than the Southern blot or PCR based techniques. Chromosome loss can also be detected cytogenetically. Mitelman (Catalog of Chromosome Aberrations in Cancer. Wiley-Liss, New York, 1995.) has compiled a catalog of over 10,000 published karyotypes of cancer cells which documents chromosome deletions as well as other changes.

Example 39. Small molecule inhibitors of variant sequences: Methylguanine Methyltransferase (MGMT) Gene VARIA 1534

The methylguanine methyltransferase gene is essential for cell growth or survival in the presence of alkylating agents

Methylguanine methyltransferase (MGMT) is a nuclear protein that repairs alkylating agent damage, specifically alkylation of the O6 position of guanine bases in genomic DNA. MGMT acts as a suicide protein in removing methyl or alkyl groups from guanine and covalently binding them to cysteine 145 of MGMT. The protein is subsequently degraded; it does not act as an enzyme. O6-benzylguanine is an inhibitor of MGMT that mimics the natural substrate, alkylated DNA; transfer of the benzyl group to cysteine 145 of MGMT inactivates the protein. Concurrent administration of O6-benzylguanine and an alkylating agent such as carmustine (BCNU) or lomustine (CCNU) renders tumor cells more sensitive to the toxic effects of the nitrosoureas by inactivataing MGMT and thereby inhibiting the tumor cells ability to repair alkylated

DNA. MGMT is thus a conditionally essential gene in the presence of nitrosoureas and other alkylating agents. The conditional essentiality of MGMT has been demonstrated in mice. Animals homozygous for disrupted MGMT genes are more than ten times as sensitive to alkylating agents as normal mice. The relative sensitivity has been measured as the LD50, the dose required to kill 50% of treated animals. (Tsuzuki, T., et al. Targeted disruption of the DNA repair methyltransferase gene renders mice hypersensitive to alkylating agent. *Carcinogenesis* 17: 1215-1220, 1996.) O6-benzylguanine is being developed as a chemosensitizing agent (with alkylating agents) for treatment of human cancer. This treatment regimen is not specific for cancer cells.

In a cancer patient with two alternative functional MGMT alleles in normal tissues and LOH at 10q23 resulting in only one copy of MGMT in cancer cells, an allele specific inhibitor of MGMT could be used to specifically sensitize cancer cells to the action of alkylating agents. Treatment would consist of the administration of the appropriate allele specific inhibitor (directed to the one allele remaining in cancer cells) plus an alkylating agent. The tumor cells would be unable to effectively repair the alkylating agent induced DNA damage, while the uninhibited allele in normal cells would be able to function. Thus normal cells, including sensitive normal cell populations such as bone marrow stem cells, would be able to tolerate higher doses of alkylating agents than cancer cells.

The MGMT gene and encoded protein are polymorphic

Four variances in human MGMT have been discovered by the inventors or reported in the literature, including three variances that affect the protein sequence. There is a C/T variance at nucleotide 255 (11% heterozygotes among 36 individuals surveyed) which does not affect the encoded protein. There is a second C/T variance at nt. 346 which results in a L84F amino acid variance (5% heterozygotes among 36 individuals surveyed). There is an A/G variance at nt. 523 which results in a I143V amino acid

variance (24% heterozygotes among 36 individuals surveyed). This variance occurs only two residues from the active site cysteine at 145. A fourth variance, G/A has been reported in the Japanese population at codon 160, GGA vs. AGA, resulting in a glycine vs. arginine amino acid variance. Fifteen percent of 40 Japanese individuals studied were heterozygotes for this variance. (Imai, Y., et al. A polymorphism at codon 160 of human O6-methylguanine-DNA methyltransferase gene in young patients with adult type cancers and functional assay. *Carcinogenesis* [London] 16:2441-24445, 1995.)

Allele specific inhibitors of MGMT

Two of the amino acid variances in MGMT, at residues 143 and 160, are near the active site of the protein. Substantial work has already been done to characterize the functional consequences of the residue 160 glycine/arginine variance. Studies of MGMT kinetics and activity have shown that the 160arginine allele is at least 20 fold more resistant to O6 benzylguanine inactivation, measured as an increase in the ED50 and or as a reduction in the production of guanine from O6-benzyl[8-3H] guanine. The 160gly and 160arg forms of MGMT were nearly equal in alkyltransferase activity in an assay that measured repair of O6-methylguanine in methylated DNA. These results demonstrate variance-specific effects of a small molecule, O6-benzylguanine, on normal (non-mutant) alleles of the conditionally essential MGMT gene. (Edara, S., et al. Resistance of the human O6-alkylguanine-DNA alkyltransferase containing arginine at codon 160 to inactivation by O6-benzylguanine. *Cancer Research* 56: 5571-5575, 1996)

Administration of O6-benzylguanine to patients who are heterozygous for the variance in their normal cells, and contain only the alternative form of the gene with a glycine residue at position 160 in their cancer cells, together with methylating or chloroethylating agents, will specifically sensitize cancer cells to the cytotoxic effects of the alkylating agents without increasing toxicity to normal cells which, since they

contain the O6-benzylguanine resistant 160arginine form of the protein, will continue to repair alkylated DNA.

There is no published data concerning the residue 143 variance, however the proximity of this variance to the active site - both in the primary sequence and upon inspection of the three dimensional structure of the bacterial AGT protein, a functional and structural homolog of human MGMT - suggests that allele specific drugs could be discovered for this variance.

The structural difference between 143isoleucine and 143valine is a hydrophobic methyl group. It is well known that most small molecule protein inhibitors interact via hydrophobic interactions. Favorable Van der Waals distances between hydrophobic groups of a substrate and a ligand are vital for high affinity interaction. One possible mechanism of allele specific inhibition would be to exploit the greater bulk of the isoleucine by finding a small molecule that fits into the active site pocket of the valine allele but has a very unfavorable Van der Waals interaction the methyl group of the isoleucine. Other schemes based on the different size and geometry of isoleucine and valine could also be effective.

One approach to identification of such inhibitors would be to make small molecule libraries in which various positions of guanine are substituted with moities of appropriate size and structure. Such libraries could then be tested in various screens of MGMT activity. The two alleles (143isoleucine and 143valine, or any of the other allele pairs of MGMT described above) would be assayed in parallel. Identification of molecules with allele specific inhibitory activity could be the basis for synthesis of additional libraries in which the moities that are best correlated with differential activity are further varied. Methods for the iterative design of high affinity or highly discriminating small molecule inhibitors are known in the art.

Libraries of restricted size can be screened for allele specific inhibitors using a combinatorial strategy based on known inhibitors of MGMT such as O6-benzyl-guanine. A library or libraries can be constructed in which substitutions are indroduced at positions C6 and N9 which have previouly been found to affect inactivation of MGMT, or at positions C2 and N8 which can be easily substituted. For example a series of 4(6)-(benzyloxy)-2,6(4)-diamino-5-(nitro or nitroso)pyrimidine derivatives and analogs in which 4(6)-benzyloxy groups were replaced with (2-, 3-, or 4 fluorobenzyl)oxy or (2-, 3-, or 4-pyridylmethyl)oxy groups have been synthesized and tested for MGMT inhibition. (Terashima I., and K. Kohda. Inhibition of human O6-alkylguanine-DNA alkyltransferase and potentiation of the cytotoxicity of chloroethylnitrosourea by 4(6)-(Benzyloxy)-2,6(4)-diamino-5-(nitro or nitroso)pyrimidine derivatives and analogues. *J Med Chem* 41: 503-508, 1998.) Substitutions at N7 have been found to be detrimental in general (Moschel, R.C. et al & Pegg, A. E., *J. Med. Chem.* 35: 4486-4491, 1992).

Combinatorial libraries can be constructed according to a published procedure (Norman, T. C. et al., A Structure-Based Library Approach to Kinase Inhibitors. *J. Am. Chem.Soc.* 118: 7430-7431, 1996) where guanine based libraries were made by anchoring a chemically modified guanine (at C6, C2, or C8) to solid supports at C2 via a glycinamide linkage or at N9 via a hydroxyethyl linkage. Chemical reactions can be carried out to introduce a library of hydrophobic substituents of different size at positions C6, C2, or C8. Hydrophobic substituents of various bulkiness and orientation can be indroduced through derivatives of O6-benzyl and O6-phenyl groups, O6-alkyl groups, N9-alkyl groups, and C2-amino-alkyl groups.

Libraries constructed as above can be screened for MGMT activity in several types of assays. Methods for bacterial expression and purification of human MGMT protein have been described (see Edara, et al., cited above). Both allelic forms of MGMT could be screened for repair of alkylated or methylated DNA by measuring transfer of tritium from a tritium labelled (methylated) DNA substrate in the

presence of various concentrations of library compounds for various times. Alternatively, library compounds could be tritiated and MGMT proteins could be screened for the rate at which they interact with (either via association or cleavage of a moiety from the compound). Other assays for MGMT activity are known in the art.

Example 41. Clinical use of variance specific inhibitors for treating cancer

Inhibitors that are the object of the present invention are designed to be administered to patients who are heterozygous for the target gene, meaning that their cells normally contain two alternative copies of the gene, one that is sensitive to inhibition by said inhibitors, and one that is not sensitive to said inhibitors. It is apparent that several such inhibitors may be developed according to this invention targeted to alternative alleles of a single target gene or to several different target genes. The inventors propose that a series of such inhibitors will be developed according to this invention.

The clinical use of this invention involves the steps of:

(a) testing normal cells from a patient to identify target genes that are heterozygous, present in two alternative forms.

(b) testing biopsy tissue from a tumor or proliferative lesion to determine whether one of the two alternative forms is eliminated due to LOH.

(c) selecting a drug for inhibition based on the presence of the sensitive allele in the tumor and the presence of an insensitive allele in normal cells

(d) administering said drug to the patient in an appropriate dose to inhibit the essential function in the cancer cell.

Testing of normal cells to identify heterozygosity of the target gene is performed

using conventional diagnostic methods that are known in the art. Normal cells are commonly derived from a blood sample, hair sample, or buccal smear. Alternatively normal cells may be obtained by cultivating primary cells such as lymphoblasts or fibroblasts in vitro. The presence of two alternative alleles may be determined by methods including allele-specific hybridization with oligonucleotides containing the variant sequences and a number of non-variant nucleotides to allow differential binding to the alternative forms of the gene or other methods known in the art using purified DNA or RNA or amplified DNA or cDNA sequences. Testing of biopsy tissue is performed by separating tumor cells or cells of the proliferative lesion to isolate a sample of cells characteristic of the proliferative lesion for analysis. This is performed by a variety of methods known in the art including manual dissection or laser assisted methods for eliminating normal cells or selecting abnormal cells. Samples of abnormal tissue, and samples of normal tissue as a control, are analyzed to identify the presence or absence of alternative forms of the target gene. The presence of two altrnative alleles may be determined by methods including allele-specific hybridization with oligonucleotides containing the variant sequences and a number of non-variant nucleotides to allow differential binding to the alternative forms of the gene or other methods known in the art using purified DNA or RNA or amplified DNA or cDNA sequences.

Selection of a drug for administration will be based on clinical trial data indicating that the drug is effective in eliminating abnormally proliferating cells and causing an improvement in the patient's clinical condition for patients who have the sensitive allele of the target gene in their pathological lesion. In one aspect of this invention, the product label will describe that the drug is indicated in patients who have only a specific allele of the target gene in their lesion and an alternative allele in their normal cells. Any such drug will be indicated only for a fraction of patients having two alternative alleles of the target gene in their normal cells and LOH. The fraction of patients who may be treated with any one drug may be determined by

multiplying the number of patients with a given cancer times the fraction of tumors exhibiting LOH of the target gene locus times the fraction of patients who will be heterozygous. For a target gene exhibiting 50% heterozygosity in the population and a 70% fraction of LOH in a specific cancer (several such examples are shown), a single inhibitor will treat ~17% of such cancers. A second compound directed against the alternative allele would treat another 17% of said cancer. In the preferred use of this invention, a panel of such drugs will be available enabling therapy with at least one such drug in most patients.

Administration of the drug to the patient ration to the patient will involve conventional means such as parenteral, oral, or intratumoral administration. The route of administration will be determined separately for each inhibitor and will be based on the bioavailability of the compound to the lesion. The compound may be administered in one or more doses as a single agent or in combination with other allele specific agents or conventional antiproliferative drugs or agents commonly used for the treatment of cancer or support of cancer patients.

Example 42.Cell Division Cycle 25C (CDC25C) - Gene VARIA10

Cdc25C is essential for cell growth

A vital regulator of cell proliferation is the protein kinase Cdc2, whose activation at the end of G2 of the cell cycle initiates mitosis. Gene disruption experiments in yeast confirm the importance of this protein, as cells lacking Cdc2 fail to progress through the cell cycle. As would be expected for such an important protein, Cdc2 activity is tightly regulated. Its activity depends on complex formation with Cyclin B, a protein that accumulates through the cell cycle and is then abruptly degraded during mitosis. Phosphorylation of Cdc2 on Tyr-15 and Thr-14 by the Wee1/Mik1

kinases maintains the Cdc2/Cyclin B complex in an inactive state until the end of G2. The dual-specificity phosphatase Cdc25C is then stimulated to dephosphorylate Cdc2 on both residues, resulting in activation of the complex. Just as Cdc2 is essential for cell growth, the regulation of its activity is essential. The best evidence for this is that the individual disruption of cdc2, cyclin B, wee 1 and cdc25 in the yeast *S. pombe* are lethal events. When cdc25 is deleted from these cells they display a phenotype consistent with their function; they grow without dividing, becoming dramatically elongated.

The human CDC25C gene and protein have variances

The CDC25C cDNA was cloned by Sadhu *et al.* (1) (Genbank accession number M34065, GI number 181075). To determine whether CDC25 is polymorphic, VARIAGENICS scanned cDNA from 32 unrelated individuals using the T4 Endonuclease VII method, which involves the cleavage of DNA heteroduplexes followed by DNA sequencing of polymorphic regions (see description of method in examples). A transversion at nucleotide 1099 (G or C) was identified (nucleotide numbering is from reference 1). This results in an amino acid difference at residue 297, with G encoding glycine and C encoding arginine. Overall, 9.4% of individuals analyzed are heterozygous. The rate of heterozygosity increases to 33.3% in Caucasians.

The human CDC25C gene maps to chromosome 5q31, a site of frequent loss of heterozygosity

Sartor *et al.* (2) mapped the human CDC25 gene to 5q31 by fluorescence in situ hybridization using the cDNA cloned by Sadhu *et al.* This mapping location was confirmed by Taviaux and Demaille (3), also using fluorescence in situ hybridization. There have been many studies of LOH on 5q, particularly the 5q21-

q22 region where the Adenomatous Polyposis Coli (APC) tumor suppressor gene lies. The most extensively studied cancers are those of the gastrointestinal tract, lung and ovary. There have been fewer studies of the 5q23-q33 region just distal to APC (where CDC25C lies), however the available data suggests that LOH occurs in this region at a frequency of ~30% in cervical cancer (4), 20-40% in colon cancer (5,6), 30-50% in ovarian cancer (7,8), up to 38% in stomach cancer (9), and 23% in testicular cancer (10). There is also evidence for LOH in head and neck, lung and liver cancers. In most of these studies only one or two markers were used. Definitive assessment of LOH frequency at the CDC25C locus will require direct analysis of the polymorphisms identified in various tumor types.

References

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Example 43. Dihydropyrimidine Dehydrogenase (DPD)

DPD is conditionally essential

Dihydropyrimidine Dehydrogenase is essential for cell survival in the presence of pyrimidine nucleotide analogs such as 5-FU and fluorodeoxyuridine. 5-fluorouracil (5-FU) and related compounds are antineoplastic drugs used in the treatment of breast, gastrointestinal, head and neck and other cancers. These drugs have widely varying clinical effects in cancer patients, ranging from induction of complete response (tumor disappearance) in some patients to severe toxicity in others. There is currently no reliable basis for predicting individual patient responses, and therefore patients receiving 5-FU must be monitored carefully for toxic reactions.

There are a variety of anabolic and catabolic pathways that affect the action of 5-FU (reviewed in Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th edition). For example, in order to exert its antiproliferative effects the pyrimidine analog 5-FU must be converted enzymatically to the nucleotide level (fluorodeoxyuridine) by phosphorylation and ribosylation; fluorodeoxyuridine is sometimes given directly because it bypasses most of these steps, and simply requires phosphorylation by thymidine kinase. The 5-fluoronucleotide is an irreversible inhibitor of thymidylate synthase, the enzyme which converts dUMP to dTMP and is required for de novo synthesis of thymidine, and hence for DNA

synthesis.

There is a three step pathway for catabolism of pyrimidines (thymine and uracil) to beta alanine. Pyrimidine analogs such as 5-FU are catabolized by the same pathway. The first and rate limiting step in this pathway is catalyzed by dihydropyrimidine dehyrogenase (DPD). DPD accounts for catabolism of as much as 90% of a 5-FU dose in normal individuals, and the half life of 5-FU in normals is ~8-20 minutes. Patients homozygous for mutant DPD alleles have been identified, a condition variously called DPD Deficiency, Hereditary Thymine-Uraciluria or Familial Pyrimidinemia. In such patients ~90% of 5-FU is excreted unchanged in the urine, and the drug has a half life longer that 2.5 hours. As a result of the drastically reduced catabolism of 5-FU the toxic effects of the drug are magnified and patients are subject to severe toxic reactions. There are reports of deaths in patients with DPD deficiency after treatment with 5-FU. Thus cell (and organism) survival in the presence of 5-FU depends on presence of functional DPD protein to transform 5-FU to the inactive dihydroxy metabolite.

This principal has also been demonstrated in cancer cells both in vitro and in vivo: cancer cells with lower DPD levels are more susceptible to the toxic effects of 5-FU. It has been suggested that measuring DPD levels would be useful for calibration of 5-FU dosage.

The DPD gene exhibits variances

We have identified four common sites of variance in DPD mRNA by screening cDNA from 36 unrelated individuals. The variant nucleotides are 166, 577, 3925 and 3937 (see DPD Variance Table; numbering is from Yokota, et al. cDNA Cloning and Chromosome Mapping of Human DIhydropyrimidine Dehydrogenase, an Enzyme Associated with 5-fluorouracil Toxicity and Congenital Thymine

Uraciluria. Journal of Biological Chemistry. 269: 23192-23196, 1994). Two of the variances in nucleotide sequence alter the amino acid coding sequence: amino acid 29 is usually cysteine but arginine alleles were also detected; cys/arg heterozygotes were found at a frequency of 11%. Residue 166 of DPD is reported to be methionine but valine is present at 166 in some alleles; 9% of the population surveyed are met/val heterozygotes. One double heterozygote was identified out of 36 patients. Both these amino acid polymorphisms are located in the N-terminal NAD/FAD binding domain of DPD. Residue 166 is located in a highly conserved domain of DPD. Two other polymorphisms are located in the 3' untranslated region of DPD, only 11 nucleotides apart.

The DPD gene maps to chromosome 1p22, a region frequently subject to LOH in different cancers

The DPD gene has been mapped to chromosome 1p22 by fluorescense in situ hybridization. LOH at 1p22 has been reported in colon, breast, and other cancers.

Allele specific inhibition of DPD to potentiate 5-FU action in cancer cells with LOH at the DPD locus

The DPD gene is polymorphic and conditionally essential in the presence of 5-FU. These properties can be exploited in a therapeutic strategy for cancer patients with LOH at the DPD locus. Specifically, in a patient with two alternative alleles for DPD in normal cells and one allele in cancer cells due to LOH, an allele specific drug can be used to sensitize cancer cells to the action of 5-FU by inhibiting its catabolism. Cancer cells (but not normal cells) would be poisoned by high levels of 5-FU due to low clearance. Normal cells, containing an uninhibited allele, would be able to catabolize DPD at close to normal levels.

Alternatively, patients heterozygous for functional and defective copies of DPD,

and in whom LOH resulted in loss of the functional allele, could be treated by 5-FU without the necessity for an allele specific inhibitor. Identification of such patients would require a test for heterozygosity at DPD and a test for LOH which could show which allele is deleted in cancer cells. Such an approach would be expected to identify patients likely to respond well to 5-FU even though they might have cancers not traditionally treated with pyrimidine analogs.

Example 44. Fanconi Anemia genes A, B, C, D, E, F, G and H (FAA, FAB, FAC, FAD, FAE, FAF, FAG, FAH)

The Fanconi Anemia genes are conditionally essential.

The Fanconi Anemia genes are essential for cell growth or survival in the presence of DNA cross linking agents. In order for cells to survive or proliferate in an abnormal environment characterized by the presence of DNA cross linking molecules such as Mitomycin C and diepoxybutane it is necessary that the cells are capable of efficiently repairing damage caused by these agents. Cells contain proteins necessary for such repair. One way such repair proteins can be identified is by absence of function in specific patients who, as a consequence, are particularly susceptible to the toxic effects of cross linking agents.

Fanconi Anemia (FA) is a hereditary disease, autosomal recessive in transmission, characterized by progressive bone marrow failure, birth defects and predisposition to malignancies. FA patients are hypersensitive to the toxicity of DNA cross linking agents. This hypersensitivity can be measured in cultured FA cells, which is one method used to establish the diagnosis of FA.

Patients heterozygous for defective FA genes are generally not hypersensitive to

DNA crosslinking agents in contrast to those that are homozygous. This suggests that treating heterozygous cancer patients with an inhibitor specific for one allele of the FA gene (and thereby reducing levels of FA protein function by up to 50% in normal cells) would be well tolerated. Inhibition of the FA allele present in cancer cells but not the alternative form present only in normal cells would make cancer cells selectively sensitive to crosslinking agents, leading to a cytotoxic antiproliferative effect. Normal cells would be able to repair damage caused by such agents, by analogy to the clinical data from patients heterozygous for defective FA genes.

The FA genes and gene products are polymorphic

Seven FA genes have been identified by complementation studies. The genes for FAA and FAC have been cloned. DNA variances have been reported in both genes. For example, Savino et al. report three variances in FAA, all of which alter the protein coding sequence. (Savino, M., et al. Mutations in the Fanconi Anemia Group A Gene (FAA) in Italian Patients. American Journal of Human Genetics 61:1246-1253, 1997.) The location of these variances is shown in the Table below, reproduced from the paper by Savino.

Variances in the FAA Gene

Polymorphic nucleotide	Alternate bases	Affected amino acid residue	Alternate amino acids	Frequency of rare allele
796	A, G	266	Thr, Ala	.29
1501	G, A	501	Gly, Ser	.40
2426	G, A	809	Gly, Asp	.30

FA genes map to chromosomes that are frequently subject to LOH in different cancers

The FAC gene maps to chromosome 9q22.3, (as do three other FA complementation

groups according to Strathdee, C.A., et al. Evidence for at least four Fanconi anaemia genes including FACC on chromosome 9. *Nature Genetics* 1: 196-198, 1992). The FAA gene maps to chromosome 16q24.3. FAD maps to 3p26-p22. All FA genes mapped so far lie in regions subject to frequent LOH. LOH affecting chromosome 9 is well documented in many cancers. For example, loss of the 9q arm is well documented in cancers such as bladder, esophagus, ovary, testis and uterus. LOH frequencies in these cancers range from 20% to 62%. LOH affecting chromosome arm 16q, particularly the 16q24 region is well documented, particularly in breast, prostate and liver cancers. For example, in six detailed studies of breast cancer in the 16q22-q24 region LOH frequencies of 40-60% have been reported. Further, 16q22 LOH has been reported in 25-90% of liver cancers, with the average around 45%. Less extensive studies of other cancer types report 16q22 LOH in 19% of bladder cancers, 20% of colon cancers, 19-27% of esophageal cancers, 25% of small cell lung cancers, 16-37% of ovarian cancers 22% of uterine cancers, and 31-50% of prostate cancers. Loss of chromosome 3p26-21 is common in lung cancer, kidney cancer, head and neck cancer and breast cancer among other cancers. Reports of >50% LOH are common in these cancer types.

Other genes conditionally essential for response to DNA cross linking agents

In a related aspect, other genes which, when defective, sensitize cells to toxic effects of DNA crosslinking agents would be amenable to the therapeutic strategy outlined above for the FA genes. Specifically, in a patient with two alternative alleles for such a gene and LOH at the relevant locus, an allele specific drug could be used to sensitize cancer cells to the action of cross linking agents. Such drugs could then be used to treat cancer patients constitutionally heterozygous for two normal alleles at the relevant locus, in whom LOH had rendered cancer cells hemizygous or homozygous for one allele. Treatment would consist in the administration of the appropriate allele specific inhibitor plus a cross linking agent or treatment to induce damage in all cells. Cancer

cells (but not normal cells) would be rendered unable to respond by inhibition of expression of the relevant repair gene. Examples of such genes are the excision repair cross complementing (ERCC) genes, twelve of which have been identified (see Target Gene Table). Defects in these genes are associated with Xeroderma Pigmentosum and Cockayne Syndrome. (Scriver, C. R. et al., The Metabolic and Molecular Bases of Inherited Disease, 7th edition, McGraw Hill, New York, 1995.)

Alternatively, patients heterozygous for functional and defective copies of such genes, and in whom LOH resulted in loss of the functional allele, could be treated by a cross-link inducing procedure without the necessity for an allele specific inhibitor. Identification of such patients would require a test for heterozygosity at the target locus and a test for LOH which could show which allele is deleted in cancer cells. Such an approach would be expected to identify patients likely to respond well to cross linking agents or procedures even though they might have cancers not traditionally treated with such agents.

Example 45. Asparagine Synthetase (AS).

Variagenics Target Gene _____

Asparagine Synthase is conditionally essential

Cells require a continuous supply of amino acids for protein biosynthesis. Cells can import amino acids from serum via amino acid transporters (the only source besides protein catabolism for the ten essential amino acids), or amino acids cells can be synthesized *de novo* by cells (only an option for the ten nonessential amino acids). The essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine. Alterations in the nutritional environment of growing cells that result in a decreased extracellular concentration of essential amino

acids cause arrested cell growth and may result in cell death.

Even a nonessential amino acid can become essential in a cell where (i) at least one enzyme required for its biosynthesis is not expressed (perhaps due to downregulation in response to an abundant extracellular supply of the amino acid), or (ii) the biosythetic pathway is blocked by an inhibitor.

Asparagine is a nonessential amino acid which is, however, essential for survival of rapidly dividing cells that are not expressing asparagine synthetase, the terminal enzyme in asparagine biosynthesis. Asparagine synthetase, considered to be a housekeeping gene, catalyzes the ATP dependent conversion of aspartic acid to asparagine in mammalian cells. A number of different cancer types do not usually express asparagine synthetase, including childhood acute leukemias. One common therapeutic used in the treatment of childhood acute lymphocytic leukemia is the enzyme L-asparaginase (purified from E. coli or Erwinia carotovora) which, upon injection, rapidly depletes serum asparagine (by hydrolysis to aspartate), thereby lowering blood levels of asparagine to undetectable levels within hours of injection. (Ohnuma, T. et al. Biochemical and Pharmacological Studies with L-Asparaginase in Man. Cancer Research 30: 2297-2305, 1970.) Leukemic cells have high rates of protein synthesis but do not express asparagine synthetase and are therefore highly vulnerable to the rapid loss of asparagine and consequent shutdown of protein synthesis. Cell death after L-asparaginase induced asparagine starvation has been shown to be apoptotic. (Bussolati, O. Characterization of Apoptotic Phenomena Induced by Treatment with L-Asparaginase in NIH3T3 Cells. Experimental Cell Research 220: 283-291, 1995.) After one or more doses leukemic cells often become resistant to L-asparaginase due to induction of asparagine synthetase activity and consequent autonomy for asparagine.

In a patient with two alternative alleles for asparagine synthetase and LOH at 7q, an

allele specific drug could be used to sensitize cancer cells to the action of L-asparaginase. Such drugs could then be used to treat cancer patients constitutionally heterozygous for two normal alleles at the asparagine synthetase locus, in whom LOH had rendered cancer cells hemizygous or homozygous for one allele. Treatment would consist in the administration of the appropriate allele specific inhibitor plus L-asparaginase to deplete the concentration of this amino acid in serum while rendering cancer cells (but not normal cells) unable to respond by upregulating asparagine synthetase.

The Asparagine Synthetase gene maps to chromosome 7q21.3, a region frequently subject to LOH in different cancers

The asparagine synthetase gene has been mapped to chromosome 7q21.3 by fluorescence in situ hybridization, following localization to 7q by analysis of somatic cell hybrids. The q21 region of chromosome 7 is subject to frequent LOH, particularly in colon, breast and prostate cancers. 7q21.3 LOH is detected in up to 50% of colon cancers, up to 37% of prostate cancers (83% of prostate cancers have LOH in the adjacent chromosome band, 7q31) and in 10-55% of breast cancers, where again, there is even more frequent LOH in 7q31. LOH at 7q21 has also been reported in uterine cancer and head and neck cancer. Several other cancer types have not yet been well studied for LOH affecting this region.

Example 46. Methionine Synthase (MS).

Variagenics Target Gene _____

Methionine Synthase is conditionally essential in dividing cells

Cells require a continuous supply of amino acids for protein biosynthesis. L-

methionine is one of ten essential amino acids. Consequently dividing cells must obtain their methionine from serum via amino acid transporter (the only source besides protein catabolism for the ten essential amino acids). Alterations in the nutritional environment of growing cells that result in a decreased extracellular concentration of essential amino acids such as methionine cause arrested cell growth and may result in cell death. Cancer cells are particularly sensitive to methionine deprivation. (Tan, Y., et al., Anticancer Efficacy of Methioninase in vivo. *Anticancer Research* 16: 3931-3936.)

The cellular requirement for methionine can be bypassed: if L-homocysteine is provided to cells it can be methylated to form methionine by the enzyme methionine synthase (MS). In this reaction the methyl group is provided by 5-methyltetrahydrofolate and MS-bound methylcobalamin serves as an intermediate methyl carrier. A second enzyme may be required for reductive activation of methionine synthase, based on complementation studies.

It occured to the inventors that the apparent antineoplastic effects of methionine deprivation could be enhanced and made tumor cell specific by preventing cells from converting endogenous homocysteine to methionine by allele specific inhibition of methionine synthase (or other enzymes required for the conversion of homocysteine to methionine; see: Scriver, C., et al., editors, The Metabolic and Molecular Basis of Inherited Disease. McGraw Hill, New York, pp. 3111-3128 and 3129-3149). This strategy would be useful in cancer patients that are heterozygous for methionine synthase (or another enzyme required for conversion of homocysteine to methionine) and who have LOH at the methionine synthase (or other) gene locus. In such patients an allele specific inhibitor of MS directed to the sole allele present in cancer cells, coupled with methionine starvation or methioninase treatment, would selectively prevent tumor cells from responding to methionine deprivation. The provision of supplemental homocysteine, which could only be converted to methionine by the

normal cells, would provide a way to amplify the differential toxicity to cancer cells. Also, the methionine analog ethionine has been shown to potentiate the effects of methionine starvation. (Poirson-Bichat, F., et al., Growth of methionine-dependent human prostate cancer (PC-3) is inhibited by ethionine combined with methionine starvation. Br. J. Cancer 75: 1605-1612.) Ethionine or similar agents could be used in conjunction with an allele specific inhibitor of methionine synthesis.

An alternative approach to allele specific therapy of cancer cells with LOH would be to target the amino acid transport system for methionine in patients heterozygous for this protein and in whom only one allele is present in cancer tissue as a result of LOH. This would result in selective methionine starvation for cancer cells. Allele specific transport inhibition could be combined with methionine starvation or methioninase treatment to enhance the cytotoxic effect.

The Methionine Synthase gene maps to chromosome 1q43, a region subject to LOH in several cancers

The MS gene has been mapped to chromosome 1q43 by fluoresence in situ hybridization. The q43 region of chromosome 1 is subject to frequent LOH particularly in colon, head and neck, ovarian and liver cancers, where LOH frequencies vary from 11 to 39%. LOH at 1q43 has also been reported in cervix, pancreas, stomach and testis cancers. Several other cancer types have not yet been well studied for LOH in this region.

Other amino acid biosynthetic enzymes are candidates for allele specific inhibition

It will be evident to one skilled in the art that strategies similar to those described above for asparagine (an essential amino acid) and methionine (a non-essential amino acid) could be undertaken for other amino acid biosynthetic enzymes. For example,

L-glutaminase has also been shown to have antiproliferative effects on mammalian cell growth. Allele specific blockade of glutamine synthesis in heterozygous patients with LOH for genes essential for glutamine synthesis could be the basis of a cancer specific therapy.

Example 47. Methylthioadenosine phosphorylase (MTAP).

Variagenics Target Gene _____

Methylthioadenosine phosphorylase can convert methylthioadenosine to methionine, an essential amino acid

Cells require a continuous supply of amino acids for protein biosynthesis. L-methionine is one of ten essential amino acids. Consequently dividing cells must obtain methionine from serum via amino acid transporter (the only source besides protein catabolism or conversion of L-homocysteine). Alterations in the nutritional environment of growing cells that result in a decreased extracellular concentration of essential amino acids such as methionine cause arrested cell growth and may result in cell death. Cancer cells are particularly sensitive to methionine deprivation. (Tan, Y., et al., Anticancer Efficacy of Methioninase in vivo. *Anticancer Research* 16: 3931-3936.)

The cellular requirement for methionine can be bypassed by conversion of L-homocysteine to methionine as discussed above. An alternative pathway for methionine synthesis is conversion of 5'-methylthioadenosine (5'-MTA) via the action of 5'-MTA phosphorylase (MTAP). (Tisdale, M.J., Methionine Synthesis from 5'-methylthioadenosine by Tumor Cells. *Biochemical Pharmacology* 32: 2915-2920.) In tissue culture experiments low concentrations of 5'-MTA can substitute for

methionine in some cell lines. Thus 5'-MTA can rescue cells from methionine deprivation.

It occured to the inventors that allele specific inhibition of MTAP in cancer patients heterozygous for MTAP and whose cancer cells have only one allele of MTAP as a consequence of LOH, in combination with methionine deprivation (methionine starvation or L-methioninase treatment) and dietary supplementation with 5'-methylthioadenosine would provide a source of convertible methionine substrate selectively useful to normal cells. Tumor cells would have no source of methionine, being unable to convert the 5'-methylthioadenosine, and hence would be selectively poisoned. This therapeutic strategy would not necessarily require an allele specific inhibitor as *all copies* of MTAP are deleted in some cancers. Such cancers should be differentially poisoned vis a vis normal cells by methionine deprivation in the presence of 5'-methylthioadenosine.

The MTAP gene maps to 9p21, a region frequently subject to LOH in many cancers

The MTAP gene has been mapped to chromosome 9p21 by physical techniques (pulsed field gel electrophoresis and yeast artificial chromosome mapping). The gene lies near the cyclin dependent kinase inhibitors p16 and p15 which are frequently reduced to one or zero copies in cancer cells. (Nobori, et al., Genomic cloning of methylthioadenosine phosphorylase: a purine metabolic enzyme deficient in multiple different cancers. *Proc. Natl. Acad. Sci. U.S.A.* 93: 6203-6208.) The p21 region of chromosome 9 is subject to frequent LOH particularly in cancers of the bladder, breast, esophagus, head and neck, kidney, lung, melanoma and ovary. The frequency of LOH in these cancers ranges from 20% to nearly 100%.

Example 48. DNA dependent protein kinase (DNA-PK) and associated factors. Variagenics Target Genes _____

DNA dependent protein kinase is conditionally essential

Cells exposed to ionizing radiation, such as gamma radiation, are damaged by base modifications and DNA strand breaks. Double strand DNA breaks are among the most lethal form of radiation damage; one such break, if unrepaired, can be cell lethal. Four complementation groups of mammalian cell mutants that are defective in repair of double strand (ds) breaks have been identified. All four complementation groups are hypersensitive to ionizing radiation. The loci for three of these groups have been shown to encode components of DNA-dependent protein kinase (DNA-PK). The fourth group is deficient in the gene encoding XRCC4, a factor that associates with and stimulates DNA Ligase IV. Ligation of ds breaks by DNA ligase IV in a cell free system in increased 7-8 fold by co-expression of XRCC4.

DNA-PK is a multiprotein complex with a DNA binding regulatory subunit, the Ku heterodimer [Ku70 (XRCC6) and Ku80, also referred to as Ku86 (XRCC5)], and a catalytic subunit, DNA-PKcs (probably XRCC7), that is activated by the regulatory subunit upon binding to DNA ds ends, with consequent expression of serine/threonine kinase activity resulting in phosphorylation of a variety of DNA binding proteins. A fourth protein called KARP-1 is expressed from the Ku80/86 locus and is also implicated in DNA-PK function.

Cells lacking any of the components of DNA-PK are exquisitely sensitive to gamma irradation. This has been demonstrated directly in mice with targeted disruption of the Ku80/86 and DNA-PKcs genes. The Ku80/86 deficient mice were also sensitive to methyl methane sulfonate, a DNA alkylating agent that induces single strand breaks and to etoposide, a topoisomerase II inhibitor. Thus the components of DNA-PK can

also be important for repair of a variety of chemically induced DNA lesions as well as ionizing radiation.

In a cancer patient with two alternative alleles for a component of DNA-PK and LOH at the heterozygous locus, an allele specific inhibitory drug could be used to sensitize cancer cells to the action of ds break inducing treatments. Such a drug could be used to treat cancer patients constitutionally heterozygous for two normal alleles at any of the DNA-PK loci in whom LOH had rendered cancer cells hemizygous or homozygous for one allele. Treatment would consist in the administration of the appropriate allele specific inhibitor plus a ds break inducing agent or procedure. The tumor cells would be unable to effectively repair ds breaks, while the uninhibited allele in normal cells would be able to function. Alternatively, patients heterozygous for functional and defective copies of genes required for repair of strand breaks, and in whom LOH resulted in loss of the functional allele, could be treated by a strand break inducing procedure without the necessity for an allele specific inhibitor. Identification of such patients would require a test for heterozygosity at the target locus and a test for LOH which could show which allele is deleted in cancer cells. Such an approach would be expected to identify patients likely to respond well to strand breaking agents or procedures (exposure to ionizing radiation) even though they might have cancers not traditionally treated with such measures.

The genes encoding constituents of DNA-PK map to chromosomes frequently subject to LOH in different cancers

The DNA-PKcs gene has been mapped to 8q11, the Ku80/86 gene to 2q11-q13 and the Ku70 gene to 22q11-q13. All three regions are subject to LOH in different cancers. LOH on 2q has been reported in lung ovary and cervical cancers at frequencies ranging from 11% to 39%. LOH for 8q has been reported in cervix, head and neck, kidney, lung, ovary, prostate and testis cancers at frequencies ranging from 20% to 50% of

cancers. LOH on 22q has been reported in brain, breast colon, head and neck, lung, ovary, pediatric and stomach cancers at frequencies ranging from 10 to 76%. Several other cancer types have not yet been well studied for LOH affecting either region.

Other proteins required for repair of DNA strand breaks are also candidates for allele specific therapy of cancer

It will be evident to one skilled in the art that strategies similar to those described above for DNA-PK could be undertaken for other proteins required for repair of DNA strand breaks. For a recent review of such proteins see: Zdzienicka, M.Z., Mammalian mutants defective in the response to ionizing radiation-induced DNA damage. *Mutation Research* 336: 203-213, 1995; Thompson, L.H. and P.A. Jeggo, Nomenclature of human genes involved in ionizing radiation sensitivity. *Mutation Research* 337: 131-134, 1995; Thacker, J. and R.E. Wilkinson, The gentic basis of cellular recovery from radiation damage: response of the radiosensitive irs lines to low-dose rate irradiation. *Radiation Research* 144: 294-300, 1995. Two other syndromes with hypersensitivity to X-rays are Diamond-Blackfan anemia and aplastic anemia (Diemen, P.C., X-ray-sensitivity of lymphocytes of aplastic- and Diamond-Blackfan-anemia patients as detected by conventional cytogentic and chromosome painting techniques. *Mutation Resarch* 373: 225-235, 1997). Recently evidence of several other genes responsible for DNA double strand break repair has been described. (Nicolas, N., Finnie, N.J., et al., Eur. J. Immunol. 26:1118-1122, 1996.) The above genes which, when defective, sensitize cells to toxic effects of DNA strand breaking agents would be amenable to the therapeutic strategy outlined above for the DNA-PK genes. Specifically, in a patient with two alternative alleles for such a gene and LOH at the relevant locus, an allele specific drug could be used to sensitize cancer cells to the action of strand breaking agents. Such drugs could then be used to treat cancer patients constitutionally heterozygous for two normal alleles at the relevant locus, in whom LOH had rendered cancer cells hemizygous or homozygous for one allele.

Treatment would consist in the administration of the appropriate allele specific inhibitor plus a strand breaking agent or treatment to induce damage in all cells. Cancer cells (but not normal cells) would be rendered unable to respond by inhibition of expression of the relevant repair gene.

Alternatively, patients heterozygous for functional and defective copies of genes required for repair of strand breaks, and in whom LOH resulted in loss of the functional allele, could be treated by a strand break inducing procedure without the necessity for an allele specific inhibitor. Identification of such patients would require a test for heterozygosity at the target locus and a test for LOH which could show which allele is deleted in cancer cells. Such an approach would be expected to identify patients likely to respond well to strand breaking agents or procedures (exposure to ionizing radiation) even though they might have cancers not traditionally treated with such measures.

Example 49. Ataxia Telangiectasia Mutated (ATM) and c-Abl Variagenics Target Gene _____

The Ataxia Telangiectasia gene is essential for cell growth or survival in the presence of ionizing radiation or DNA damaging molecules

In order for cells to survive or proliferate in the presence of ionizing radiation (IR) or radiomimetic chemicals it is necessary that they are capable of efficiently repairing IR induced damage. Cells contain proteins necessary for such repair. One way such proteins can be identified is by their absence in specific patients who are particularly susceptible to the toxic effects of IR.

Ataxia Telangiectasia (AT) is a genetically transmitted autosomal recessive disorder characterized by variable degrees of immunodeficiency, telagiectasia (small blood vessels growing near the surface of the skin or eye), cerebellar ataxia (loss of balance due to abnormal development of the cerebellum) and increased sensitivity to both ionizing radiation and radiomimetic drugs, including bleomycin; AT cells are killed by lower doses of ionizing radiation or radiomimetic drugs than normal cells. Further, heterozygotes for mutant and normal AT alleles have radiation sensitivity close to that of homozygous normals. Therefore cancer cells from individuals heterozygous for null alleles of the AT gene (called ATM) should be highly susceptible to radiation therapy when only the deficient AT allele remains in cancer cells due to LOH, compared to normal cells from the same patients. Such patients could be treated by a DNA damage inducing procedure without the necessity for an allele specific inhibitor. Identification of such patients would require a test for heterozygosity at the target locus and a test for LOH which could show which allele is deleted in cancer cells. Such an approach would be expected to identify patients likely to respond well to strand breaking agents or procedures (such as exposure to ionizing radiation) even though they might have cancers not traditionally treated with such measures. In a related aspect, this approach is applicable to heterozygotes for other genes associated with ATM-mediated radiosensitivity. One such protein is the c-Abl protein tyrosine kinase, which binds to the ATM protein and regulates its function. c-Abl is known to be important in the stress response to ionizing radiation. One of its functions is activation of stress activated protein kinases (SAPKs) after irradiation or exposure to alkylating agents such as *cis*-platinum or mitomycin C, a response that is defective in ATM cells. Correction of the SAPK activation defect in ATM cells by non-mutant ATM cDNA suggests that the ATM - c-Abl interaction is necesary for the DNA damage response. (Kharbanda, S., et al. *Nature* 376: 785-788, 1995.)

In a cancer patient with two alternative functional alleles for a component of ATM and LOH at the ATM locus, an allele specific inhibitory drug could be used to sensitize

cancer cells to the action of DNA damage inducing treatments such as ionizing radiation or radiomimetic drugs. Such an allele specific drug could be used to treat cancer patients constitutionally heterozygous for two normal ATM alleles in whom LOH had rendered cancer cells hemizygous or homozygous for one allele. Treatment would consist of the administration of the appropriate allele specific inhibitor plus a DNA damage inducing treatment or procedure. The tumor cells would be unable to effectively the DNA damage, while the uninhibited allele in normal cells would be able to function. A similar approach could be taken to

The ATM gene is polymorphic

The ATM cDNA is 9.58 kb. Several likely polymorphisms have been identified, although population studies have not yet been performed to determine allele frequencies. One of the reported polymorphisms, an ATG to ATA change in codon 847, results in a methionine vs. isoleucine difference. Thus ATM is potentially targetable at the DNA, RNA and protein levels. It is likely that additional variances will be identified with broader population surveys and computational variance detection.

The ATM gene maps to chromosome 11q23 and the c-Abl gene maps to 9q34.1, two regions of high frequency LOH in different cancer types

Chromosome 9q34 is lost in a high fraction of bladder, esophagus, ovary, head & neck and testis cancers (17 - 76%) and in a lesser fraction of breast, liver and prostate cancers and leukemias. Chromosome 11q23 is lost in brain, cervix, esophagus, breast, kidney, colon, stomach, head & neck and lung cancers at frequencies ranging from 16% to 100%.

Other proteins required for repair of DNA damage are also candidates for allele specific therapy of cancer

It will be evident to one skilled in the art that strategies similar to those described above for ATM and c-Abl could be undertaken for other proteins required for the stress response to DNA damaging agents, such as other stress activated protein kinases or downstream effector proteins.

Methylguanine Methyltransferase (MGMT)
Gene VARIA 1534

The methylguanine methyltransferase gene is essential for cell growth or survival in the presence of alkylating agents

Methylguanine methyltransferase (MGMT) is a suicide protein that repairs alkylating agent damage, specifically alkylation of the ^{6}O position of guanine. Alkyl groups are covalently bound to an active site cysteine (residue 145) of MGMT, thereby irreversibly inactivating the protein. ^{6}O-benzylguanine is an analog inhibitor of MGMT that, by inactivating MGMT, renders tumor cells more sensitive to the toxic effects of methylating and chloroethylating agents. MGMT is thus a conditionally essential gene in the presence of such drugs. ^{6}O-benzylguanine is being developed as a chemosensitizing agent.

In a cancer patient with two alternative functional MGMT alleles an allele specific inhibitory drug could be used to sensitize cancer cells to the action of alkylating agents. Such an allele specific drug could be used to treat cancer patients constitutionally heterozygous for two normal MGMT alleles in whom LOH had rendered cancer cells hemizygous or homozygous for one allele. Treatment would consist of the administration of the appropriate allele specific inhibitor plus an alkylating agent. The tumor cells would be unable to effectively repair the alkylating agent induced DNA damage, while the uninhibited allele in normal cells would be able to function.

The MGMT gene is polymorphic

Several variances have been reported in human MGMT, or discovered by Variagenics, including three protein polymorphisms. There is a silent C/T variance at position 255 (11% heterozygotes among 36 individuals surveyed), another C/T variance at nt. 346

which results in a L84F amino acid variance (5% heterozygotes), an A/G variance at nt. 523 which results in a I143V amino acid variance (24% heterozygotes). A variance has been reported in Japanese at codon 160, GGA vs. AGA, converting glycine to arginine. 15% of the population studied were heterozygotes.

The alteration of glycine 160 to arginine reduced the inactivation by O6-benzylguanine with an approximately 20 fold increase in the IC50 concentration. These results demonstrate variance-specific effects of a small molecule, O6-benzylguanine, on normal (non-mutant) alleles of the conditionally essential MGMT gene.

Administration of O6 benzylguanine to patients who are heterozygous for the residue 160 gly/arg variance in their normal cells, and contain only the form of the gene with a glycine residue at position 160 in their cancer cells, together with methylating or chloroethylating agents for chemotherapy, will be specifically toxic to cancer cells without increasing toxicity to normal cells.

References

1. Imai, Y, *Carcinogenesis* (1995), 16:2441-24445

2. Edara, S. (1996) Resistance of the human O6-alkylguanine-DNA alkyltransferase containing arginine at codon 160 to inactivation by O6-benzylguanine. *Cancer Research* 56, 5571-5575.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well

adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The groups of genes and the particular genes described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. For example, those skilled in the art will readily recognize that the methods and inhibitors can utilize a variety of different target genes within the groups described. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Thus, additional embodiments are within the scope of the invention and within the following claims.

CLAIMS

What we claim is:

1. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene encodes a product required for cell proliferation;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

2. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of

said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

3. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

4. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles

or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

5. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

6. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

7. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene is located on a high frequency LOH chromosomal region;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

8. The method of claim 7, wherein said gene is located on a chromosomal arm which has a frequency of allele loss of at least 15% in a cancer.

9. The method of claim 7, wherein said gene is located in proximity to a chromosomal marker which undergoes LOH at a frequency of at least 10% in a cancer.

10. The method of claim 7, wherein said gene is located in proximity to a tumor suppressor gene which undergoes LOH at a frequency of at least 10% in a cancer.

11. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a gene vital for cell growth or viability, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene, wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

12. The method of claim 11, wherein said gene is located on a high frequency LOH chromosomal region.

13. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth, wherein said gene encodes a product required for cell proliferation, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

14. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth,wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival, said gene has at least two alternative

alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

15. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth, wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

16. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth, wherein said gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

17. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth, wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

18. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth,wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

19. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth, wherein said gene is located on a high frequency LOH chromosomal arm region, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

20. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a gene vital for cell viability or cell growth, wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

21. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene encodes a product required for cell proliferation; and

a pharmaceutically acceptable carrier or excipient.

22. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival; and

a pharmaceutically acceptable carrier or excipient.

23. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival; and

a pharmaceutically acceptable carrier or excipient.

24. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival; and

a pharmaceutically acceptable carrier or excipient.

25. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival; and

a pharmaceutically acceptable carrier or excipient.

26. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures; and

a pharmaceutically acceptable carrier or excipient.

27. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene is located on a high frequency LOH chromosomal arm region; and

a pharmaceutically acceptable carrier or excipient.

28. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of an essential gene in a population, wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene; and

a pharmaceutically acceptable carrier or excipient.

29. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene encodes a product required for cell proliferation;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

30. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

31. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

32. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

33. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival;

(b) screening to identify an inhibitor which inhibits said at least one but less

than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

34. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

35. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene is located on a high frequency LOH chromosomal arm region;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

36. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a gene having at least two alternative alleles, comprising the steps of:

(a) identifying a gene vital to cell viability or cell growth that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell, and wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in which cancerous cells have only the allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene.

37. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene encodes a product required for cell proliferation; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

38. The method of claim 37, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

39. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

40. The method of claim 39, wherein the cells of said precancerous condition are

not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

41. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

42. The method of claim 41, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in

cells of said precancerous condition.

43. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

44. The method of claim 43, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

45. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are

heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

46. The method of claim 45, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

47. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene encodes a product required to maintain the integrity and function of cellular and subcellular structures; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

48. The method of claim 47, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

49. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene is located on a high frequency LOH chromosomal arm region; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

50. The method of claim 49, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for

each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

51. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of a first essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells, and said first gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

52. The method of claim 51, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

b. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different essential gene, and wherein said patient is heterozygous for each targeted essential gene and each targeted essential gene has undergone LOH in cells of said precancerous condition.

53. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at

least one but less than all allelic forms of said gene present in a population,

wherein said gene encodes a product required for cell proliferation, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

54. The method of claim 53, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

55. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

56. The method of claim 55, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

57. A method for treating a patient suffering from a cancer, wherein said patient

is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

58. The method of claim 57, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

59. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

60. The method of claim 59, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

61. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

62. The method of claim 61, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

63. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

64. The method of claim 63, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are

heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

65. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene is located on a high frequency LOH chromosomal arm region, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

66. The method of claim 65, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

67. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a gene vital for cell growth or viability, comprising the step of:

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene, said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said

patient.

68. The method of claim 67, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular gene essential for cell growth or viability; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

69. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene encodes a product required for cell proliferation, and wherein said inhibitor is less active on at least one other allele of said gene.

70. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival, and wherein said inhibitor is less active on at least one other allele of said gene.

71. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival, and wherein said inhibitor is less active on at least one other allele of said gene.

72. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene encodes a product required to maintain cellular proteins at levels compatible with cell growth or survival, and wherein said inhibitor is less active on at least one other allele of said gene.

73. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival, and wherein said inhibitor is less active on at least one other allele of said gene.

74. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures, and wherein said inhibitor is less active on at least one other allele of said gene.

75. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene is located on a high frequency LOH chromosomal arm region, and wherein said inhibitor is less active on at least one other allele of said gene.

76. A method of inhibiting growth of a cell comprising the step of:

administering at least one inhibitor active on an allele of a gene vital for cell viability or growth,

wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene, and wherein said inhibitor is less active on at least one other allele of said gene.

77. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

identifying a patient heterozygous for a said gene encoding a product required for cell proliferation,

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

78. The method of claim 77, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

79. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene encoding a product required for cell proliferation,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

80. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

identifying a patient heterozygous for a said gene encoding a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival,

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

81. The method of claim 80, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

82. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene encoding a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

83. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the steps of:

identifying a patient heterozygous for a said gene encoding a product required to maintain organic compounds at levels compatible with cell growth or survival;

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

84. The method of claim 83, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

85. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene encoding a product required to maintain organic compounds at levels compatible with cell growth or survival,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

86. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the steps of:

identifying a patient heterozygous for a said gene encoding a product required to maintain cellular proteins at levels compatible with cell growth or survival ;

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

87. The method of claim 86, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

88. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene encoding a product required to maintain cellular proteins at levels compatible with cell growth or survival ,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

89. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the steps of:

identifying a patient heterozygous for a said gene encoding a product required to maintain cellular nucleotides at levels compatible with cell growth or survival ;

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

90. The method of claim 89, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

91. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene encoding a product required to maintain cellular nucleotides at levels compatible with cell growth or survival,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

92. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the steps of:

identifying a patient heterozygous for a said gene encoding a product required to maintain the integrity and function of cellular and subcellular structures ;

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

93. The method of claim 91, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

94. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a

said gene encoding a product required to maintain the integrity and function of cellular and subcellular structures,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

95. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the steps of:

identifying a patient heterozygous for a said gene located on a high frequency LOH chromosomal arm region ;

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

96. The method of claim 95, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

97. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene located on a high frequency LOH chromosomal arm region,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

98. A method of identifying a potential patient for treatment with an inhibitor

active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the steps of:

identifying a patient heterozygous for a said gene which has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene;

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

99. The method of claim 98, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

100. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a gene vital for cell growth or viability, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene which has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

101. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene encodes a product required for cell proliferation, wherein said portion comprises a sequence variance site, and wherein said probe

hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

102. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene encodes a product required to maintain inorganic ions and vitamins at levels compatible with cell growth or survival, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

103. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene encodes a product required to maintain organic compounds at levels compatible with cell growth or survival, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

104. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene encodes a product required to maintain cellular

proteins at levels compatible with cell growth or survival, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

105. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene encodes a product required to maintain cellular nucleotides at levels compatible with cell growth or survival, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

106. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene encodes a product required to maintain the integrity and function of cellular and subcellular structures, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

107. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or

viability,

wherein said gene is located on a high frequency LOH chromosomal arm region, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

108. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a gene vital for cell growth or viability,

wherein said gene has at least two sequence variances which occur at frequences such that at least 10% of a population is heterozygous for said gene, wherein said portion comprises a sequence variance site, and wherein said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

109. The method ,inhibitor, pharmaceutical composition, or nucleic acid probe of any of claims 1, 13, 21, 29, 37, 53, 69, 77, and 101, wherein said gene is selected from the group consisting of 14-3-3 Protein TAU, CCNA(G2/Mitotic-Specific Cyclin A), CCNB1(G2/Mitotic-Specific Cyclin B1), CCND1(G1/S-Specific Cyclin D1), CCND2(G1/S-Specific Cyclin D2), CCND3(G1/S-Specific Cyclin D3), Cell division control protein 16, Cell division cycle 2, G1 to S and G2 to M, Cell division cycle 25A, Cell division cycle 25B, Cell division cycle 25C, Cell division cycle 27, Cell division-associated protein BIMB, Cyclin A1(G2/Mitotic-Specific Cyclin A1), Cyclin C (G1/S-Specific Cyclin C), Cyclin G1(G2/Mitotic-Specific Cyclin G), Cyclin G2 (G2/Mitotic-Specific Cyclin G), Cyclin H, Cyclin H Assembly, GSPT1(G1 to S phase transition 1), Mitotic MAD2 Protein, MRNP7, RANBP1(RAN binding protein 1), WEE1, Cell Division Protein Kinase 4, CDC28 protein kinase 1, CDC28 protein

kinase 2, M-Phase inducer phosphatase 2, M-phase phosphoprotein, mpp6, PPP1ca(Protein phosphatase 1, catalytic subunit, alpha isoform), STM7-LSB, CENP-F kinetochore protein, Centromere autoantigen C, Centromere protein B (80kD), Centromere protein E (312kD), CHC1(Chromosome condensation 1), Chromatin assembly factor-I p150 subunit, Chromatin assembly factor-I p60 subunit, Chromosome segregation gene homolog CAS, HMG1(High-mobility group (nonhistone chromosomal) protein 1), Minichromosome Maintenance (MCM7), Mitotic centromere-associated kinesin, RMSA1(Regulator of mitotic spindle assembly 1), and SUPT5h(Chromatin structural protein homolog (SUPT5H)).

110. The method ,inhibitor, pharmaceutical composition, or nucleic acid probe of any of claims 2, 14, 22, 30, 39, 55, 70, 80, and 102, wherein said gene is selected from the group consisting of PMCA1 (Calcium Pump), PMCA2 (Calcium Pump), PMCA3 (Calcium Pump), PMCA4 (Calcium Pump), ATP2b1 (Calcium-Transporting ATPase Plasma Membrane), ATP2b2 (Calcium-Transporting ATPase Plasma Membrane), ATP2b4 (Calcium-Transporting ATPase Plasma Membrane), ATP5b (ATP Synthase Beta Chain, Mitochondrial Precursor), Chloride Conductance Regulatory Protein ICLN, H-Erg (Potassium Channel Protein EAG), Nuclear Chloride Ion Channel Protein (NCC27), SCN1b(Sodium Channel, Voltage-Gated, Type I, Beta Polypeptide), Two P-Domain K+ Channel TWIK-1, VDAC2 (Voltage-Dependent Anion-Selective Channel Protein 2), ATP1b1 (Sodium/Potassium-Transporting ATPase Beta-1 Chain), ATP1b2 (Sodium/Potassium-Transporting ATPase Beta-2 Chain), ATPase, Ca++ transporting, plasma membrane 4, ATPase, Ca++ transporting, plasma membrane 2, ATPase, Na+/K+ transporting, alpha 1 polypeptide, ATPase, Na+/K+ transporting, alpha 3 polypeptide, ATPase, Na+/K+ transporting, beta 1 polypeptide, ATPase, Na+/K+ transporting, beta 2 polypeptide, Na+,K+ ATPase, 1 Subunit, Na+,K+ ATPase, 2 alpha, Na+,K+ ATPase, 3 beta, SLC9a1(Solute carrier family 9 (sodium/hydrogen exchanger)), Solute carrier family 4, anion exchanger, member 1, Solute carrier family 4, anion

exchanger, member 2, Solute carrier family 9 (sodium/hydrogen exchanger),Passive transporters, MaxiK Potassium Channel Beta Subunit, Chloride Channel 2, Chloride Channel Protein (CLCN7), TRPC1 (Transient Receptor Potential Channel 1), Potassium Channel Kv2.1, ATP5d(ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit), ATP5f1(ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b), ATP5o(ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit), ETFa(Electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)), ETFb(Electron-transfer-flavoprotein, beta polypeptide), Nadh-ubiquinone oxidoreductase 13 kd-B subunit, Nadh-ubiquinone oxidoreductase 39 kD subunit precursor, NADH-Ubiquinone oxidoreductase 75 kD subunit precursor, NADH-Ubiquinone oxidoreductase MFWE subunit, NDUFV2(NADH dehydrogenase (ubiquinone) flavoprotein 2 (24kD)), Ubiquinol-cytochrome c reductase complex 11 kD, ATP Synthase Alpha Chain, NADH dehydrogenase-ubiquinone Fe-S protein 8, 23 kDa subunit, Ascorbic Acid (transporter), Folate Binding Protein, Folate receptor 1 (adult), Nicotinamide (transporter), Pantothenic Acid transporter, Riboflavin (transporter), SCL19A1 (Solute Carrier Family 19, Member1), Solute carrier family 19 (folate transporter), member 1, Thiamine, B6, B12 (transporter), ATP7b (Copper-Transporting ATPase 2), Ceruloplasmin (ferroxidase), Ceruloplasmin receptor (Copper Transporter), Copper Transport Protein HAH1, Molybdenum, Selenium, Tranferrin Receptor (Iron Transporter), Zinc Transporter, and mitochondrial import receptor subunit TOM20.

111. The method ,inhibitor, pharmaceutical composition, or nucleic acid probe of 3, 25, 23, 31, 41, 57, 71, 83, and 103, wherein said gene is selected from the group consisting of GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, GLUT6, Solute carrier family 5 (sodium/glucose cotransporter), Solute carrier family 2 (facilitated glucose transporter), member 2, Solute carrier family 2 (facilitated glucose transporter) member 5, Solute carrier family 3 member 1, System b,(Na+ independent), System y,(Na+ independent), ATRC1(Catioinc), LEUT(Leucine Transporter),

SLC1A1(Solute Carrier Family 1, Member 1), Solute carrier family 16 (monocarboxylic acid transporters), ACO1(Aconitase 1), ACO2(Aconitase 2, mitochondrial), Acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain, Acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain, Acyl-Coenzyme A dehydrogenase, long chain, Acyl-Coenzyme A dehydrogenase, very long chain, aKGD (alpha ketoglutaratedehydrogenase), ALD-a (Aldolase), ALD-b (Aldolase), ALD-c (Aldolase), CS (Citrate Synthetase), Dihydrolipoamide S-succinyltransferase, DLAT(Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)), DLD(Dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex)), E1k (Oxoglutarate dehydrogenase), E2k (Dihydrolipoamide S-succinyltransferase), E3 (Dihydrolipoyl Dehydrogenase), ENO1(Enolase 1,alpha), ENO2(Enolase 2), ENO3(Enolase 3), Enolase 2, (gamma, neuronal), Enolase 3, (beta, muscle), FH(Fumarate hydratase), G3PDH (Glyceraldehyde-3-Phosphate Dehydrogenase), G6PD (Glucose-6-Phosphate Dehydrogenase), Glucose-6-phosphate dehydrogenase, HK1 (Hexokinase 1), HK2 (Hexokinase 2), HK3 (Hexokinase 3), IDH1(Isocitrate dehydrogenase 1 (NADP+), soluble), IDH2(Isocitrate dehydrogenase 2 (NADP+), mitochondrial), MDH1(Malate dehydrogenase 1, NAD (soluble)), MDH2(Malate dehydrogenase 1, NAD (mitochondrial)), NAD(H)-specific isocitrate dehydrogenase alpha subunit, Oxoglutarate dehydrogenase (lipoamide), PDHB (Pyruvate Dehydrogenase), PDHB(Pyruvate dehydrogenase (lipoamide) beta), PDK4 (Pyruvate dehydrogenase kinase, isoenzyme 4), PFKL(Phosphofructokinase), PGI (Phosphoglucoisomerase), PGKa (Phosphoglyceromutase), PGKb (Phosphoglyceromutase), PGM1 (Phosphoglyceromutase), PGM2 (Phosphoglyceromutase), PGM3 (Phosphoglyceromutase), PGM4 (Phosphoglyceromutase), Phosphofructokinase, muscle, Phosphoglucomutase 1, Phosphoglycerate kinase 1, PK1 (Pyruvate Kinase), PK2 (Pyruvate Kinase), PK3 (Pyruvate Kinase), Pyruvate dehydrogenase kinase isoenzyme 2 (PDK2), Pyruvate kinase, liver, Pyruvate kinase, muscle,

SDH1(Succinate dehydrogenase, iron sulphur (Ip) subunit), SDH2(Succinate dehydrogenase 2, flavoprotein (Fp) subunit), TKT(Transketolase (Wernicke-Korsakoff syndrome)), TPI (Trisephosphate Isomerase), Asparagine Synthetase, Aminoacylase-1, Aminoacylase-2, ACAC (Acetyl CoA Carboxylase Beta), ACAC (Acetyl CoA Carboxylase), ACADSB(Acyl-coA dehydrogenase), Mevalonate kinase, Phosphomevalonate kinase, Aspartoacylase, Ornithine decarboxylase 1, Short-acyl-CoA dehydrogenase, Medium acyl-CoA dehydrogenase, Long acyl-CoA dehydrogenase, Isovalveryl CoA dehydrogenase, 2-methyl branched chain, Adenosine Deaminase, Purine-nucleoside phosphorylase, Guanine Deaminase, Xanthine Oxidase, ITM1 (Integral Transmembrane Protein), GFPT (Glutamine-Fructose-6-Phosphate Transaminase), Heparan, Polypeptide N-Acetyltransferase, ACAA(Acetyl-Coenzyme A acyltransferase), Lysophosphatidic acid acyltransferase-alpha, Lysophosphatidic acid acyltransferase-beta, FNTa (Farnesyltransferase Alpha Subunit), FNTb (Farnesyltransferase Beta Subunit), NMT1 (N-myristoyltransferase), Calcineurin A, Calcineurin B, Calreticulin Precursor, Phosphatase 2b, PPP3ca(Protein phosphatase 3 , catalytic subunit), SNK Interacting 2-28(Calcineurin B Subunit), Protein Kinase C, PRKCA(Protein kinase C, alpha), PRKCB1(Protein kinase C, beta 1), PRKCD(Protein kinase C, delta), PRKCM(Protein kinase C, mu), PRKCQ(Protein kinase C-theta), PRKCSH(Protein kinase C substrate 80K-H), Geranylgeranyl, Geranylgeranyltransferase (Type I Beta), GGTB (Geranylgeranyltransferase), Geranylgeranyltransferase (Type II Beta-Subunit), Gdp Dissociation Inhibitors, GDI Alpha (RAB GDP Dissociation Inhibitor Alpha), and Rab Gdp (RAB GDP Dissociation Inhibitor Alpha).

112. The method, inhibitor, pharmaceutical composition, or nucleic acid probe of any of claims 4, 16, 24, 32, 43, 59, 72, 86, and 104, wherein said gene is selected from the group consisting of GOT(Glutamic-oxaloacetic transaminase 2), GOT1(Glutamic-oxaloacetic transaminase 1), PYCS(Pyrroline-5-carboxylate synthetase), Tyrosine aminotransferase, AARS, CARS, DARS, EPRS, FARS,

GARS, HARS, IARS, KARS, LARS, MARS, NARS, QARS , RARS, SARS, TARS, VARS, WRS, YARS, Ribosomal Protein L11, Ribosomal Protein L12, Ribosomal Protein L17, Ribosomal Protein L18, Ribosomal Protein L18a, Ribosomal Protein L19, Ribosomal Protein L21, Ribosomal Protein L22, Ribosomal Protein L23, Ribosomal Protein L23a, Ribosomal Protein L25, Ribosomal Protein L26, Ribosomal Protein L27, Ribosomal Protein L27a, Ribosomal Protein L28, Ribosomal Protein L29, Ribosomal Protein L30, Ribosomal Protein L31, Ribosomal Protein L32, Ribosomal Protein L35, Ribosomal Protein L35a, Ribosomal Protein L36a, Ribosomal Protein L39, Ribosomal Protein L4, Ribosomal Protein L41, Ribosomal Protein L44, Ribosomal Protein L6, Ribosomal Protein L7, Ribosomal Protein L7a, Ribosomal Protein L8, Ribosomal Protein L9, Ribosomal Protein P1, Ribosomal Protein S10, Ribosomal Protein S11, Ribosomal Protein S13, Ribosomal Protein S14, Ribosomal Protein S15, Ribosomal Protein S15A, Ribosomal Protein S16, Ribosomal Protein S17, Ribosomal Protein S17A, Ribosomal Protein S17B, Ribosomal Protein S18, Ribosomal Protein S20, Ribosomal Protein S20A, Ribosomal Protein S20B, Ribosomal Protein S21, Ribosomal Protein S23, Ribosomal Protein S25, Ribosomal Protein S26, Ribosomal Protein S28, Ribosomal Protein S29, Ribosomal Protein S3, Ribosomal Protein S3A, Ribosomal Protein S4, Ribosomal Protein S4X, Ribosomal Protein S4Y, Ribosomal Protein S5, Ribosomal Protein S6, Ribosomal Protein S7, Ribosomal Protein S8, Ribosomal Protein S9, Initiation of polypeptide polymerization, eIF-2 (Eukaryotic initiation factor), eIF-2-associated p67(Eukaryotic initiation factor), eIF-2A(Eukaryotic initiation factor), eIF-2Alpha(Eukaryotic initiation factor), eIF-2B(Eukaryotic initiation factor), eIF-2B-Gamma(Eukaryotic initiation factor), eIF-2Beta(Eukaryotic initiation factor), eIF-3 p110(Eukaryotic initiation factor), eIF-3 p36(Eukaryotic initiation factor), eIF-4A(Eukaryotic initiation factor), eIF-4C(Eukaryotic initiation factor), eIF-4E(Eukaryotic initiation factor), eIF-4Gamma(Eukaryotic initiation factor), eIF-5(Eukaryotic initiation factor), eIF-5A, Eukaryotic peptide chain release factor subunit 1, P97(Eukaryotic initiation factor), eEF1A2(Eukaryotic elongation factor),

eEF1D(Eukaryotic elongation factor), eEF2(Eukaryotic elongation factor), eIF4A2 (Eukaryotic initiation factor), KIAA0031(Elongation factor 2), KIAA0219(Putative translational activator C18G6.05C), Factor 1-Alpha 2(Eukaryotic translation elongation factor 1 alpha 2), Cis-Trans Isomerase, DNAj Protein Homolog 1, DNAj Protein Homolog 2, DNAJ Protein homolog HSJ1, T-Complex, Aspartylglucosaminidase, T-Complex 1, Alpha, T-Complex 1, Epsilon, T-Complex 1, Gamma, T-Complex 1, Theta, T-Complex 1, Zeta, 26S Protease regulatory subunit 4, Alpha-2-Macroglobulin, Calpain 1, Large, CLPP(ATP-Dependent CLP protease proteolytic subunit), KIAA0123 (Mitochondrial processing peptidase alpha subunit), MMP7, Proteasome Beta 6, Proteasome Beta 7, Proteasome C13, Proteasome C2, Proteasome C7-1, Proteasome inhibitor hPI31 subunit, Proteasome P112, Proteasome P27, Proteasome P55, Enzyme E2-17 Kd(Cyclin-selective ubiquitin carrier protein), ISOT-3(Ubiquitin carboxyl-terminal hydrolase T), ORF (Ubiquitin carboxyl-terminal hydrolase 14), PGP(Ubiquitin carboxyl-terminal hydrolase isozyme L1), UBA52(Ubiquitin A-52 residue ribosomal protein fusion product 1), Ubiquitin carboxyl-terminal hydrolase 3, Ubiquitin carboxyl-terminal hydrolase isozyme L3, Ubiquitin carboxyl-terminal hydrolase T, Ubiquitin carrier protein (E2-EPF), Ubiquitin fusion-degradation protein (UFD1L), Ubiquitin Hydrolase, Ubiquitin-conjugating enzyme E2I, SEC23(Protein transport protein SEC23), SEC23A(Protein transport protein SEC23), SEC7(Protein transport protein SEC7), SEC61 (Beta Subunit), and LDLR (LDL receptor).

113. The method, inhibitor, pharmaceutical composition, or nucleic acid probe of any of claims 5, 17, 25, 33, 45, 73, 89, and 105, wherein said gene is selected from the group consisting of Adenylate Kinase-2, Adenylosuccinate synthetase, Adenylosuccinate Lyase, DPRT (ADP-Ribosyltransferase), ADSL (Adenylosuccinate lyase/AMP synthetase), ADSS (Adenylosuccinate Synthetase), CAD PROTEIN, CTP Synthetase, CTPS(CTP synthetase), Cytidine Triphosphate Synthetase, GARS (Phosphoribosylglycinamide synthetase), GART (Phosphoribosylglycinamide

formyltransferase), GART(Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase), GMP Synthetase, IMP Cyclohydrolase, IMP dehydrogenase, IMPDH1(IMP (inosine monophosphate) dehydrogenase 1), IMPDH2(IMP (inosine monophosphate) dehydrogenase 2), Phosphoribosyl diphosphotransferase, Phosphoribosylaminoimidazolecarboxamide formyltransferase, Phosphoribosylformylglycinamide synthetase, Phosphoribosylglycinamide carboxylase, Phosphoribosylglycinamide-succinocarboxamide synthetase, PPAT (Amidophoribosyltransferase), PPAT(Phosphoribosyl pyrophosphate amidotransferase), Ribonucleoside-diphosphate reductase M1 chain, Ribonucleoside-diphosphate reductase M2 chain, Thymidine Kinase, Thymidylate Synthase, UMK(Uridine kinase), UMPK (Uridine monophosphate kinase), UMPS(Uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase)), Uridine Phosphorylase, DNA Origin Recognition Complex, ORC1, ORC2, ORC3, ORC4, ORC5, ORC6, ORC Regulators, CDC6, CDC7, CDC1, DNA Polymerization, DNA Polymerases, Adprt (NAD(+) ADP-Ribosyltransferase), DNA Polymerase Alpha-Subunit, DNA Polymerase Delta, POLa(DNA Polymerase Alpha/Primase Associated Subunit), POLb(DNA Polymerase Beta Subunit), POLd1(Polymerase (DNA directed), Delta 1, Catalytic Subunit), POLd2(Polymerase (DNA directed), Delta 2), POLE(Polymerase (DNA directed)), POLg (DNA Polymerase Gamma Subunit), Terminal Transferase (DNA Nucleotidylexotransferase), Activator 1 36 Kd, CDC46 (DNA Replication Licensing Factor), CDC47 (DNA Replication Licensing Factor CDC47), DNA Topoisomerase III, DRAP1 (DNA Replication Licensing Factor MCM3), KIAA0030 Gene (Cell Division Control Protein 19), KIAA0083 Gene (DNA Replication Helicase DNA2), MCM3 (DNA Replication Licensing Factor MCM3), PCNA (Proliferating Cell Nuclear Antigen), PRIM1 (DNA Primase 49 kD Subunit), PRIM2 (DNA Primase), PRIM2a (DNA Primase 58 kD Subunit), PRIM2b (DNA Primase), RECa (Replication Protein A 14 kD Subunit), RFC1 (Replication Factor C (activator 1) 1),

RFC2 (Replication Factor C 2), RFC3 (Replication Factor C (activator 1) 3), RFC4 (Replication Factor C, 37-kD subunit), RFC5 (Replication Factor C), RPA1 (Replication protein A1 (70kD)), RPA2 (Replication protein A2 (32kD)), RPA3 (Replication protein A3 (14kD)), TOP1 (DNA Topoisomerase I), TOP2a (Topoisomerase (DNA) II Alpha (170kD)), TOP2b (Topoisomerase (DNA) II Beta (180kD)), CHL1(CHL1-Related Helicase), DNA Helicase II, Mi-2(Chromodomain-Helicase- DNA-Binding Protein CHD-1), RECQL (ATP-Dependent DNA Helicase Q1), Smbp2 (DNA-Binding Protein SMUBP-2), H1(0) (Histone H5A), Histone H1d, Histone H1x, Histone H2a.1, Histone H2a.2, Histone H2b.1, Histone H4, SLBP (Histone Hairpin-Binding Protein), TATA-binding Complex, Small Nuclear RNA-Activating Complex, Polypeptide 1, 43KD (SNAPC1), Small Nuclear RNA-Activating Complex, Polypeptide 2, (SNAPC2), Small Nuclear RNA_Activating Complex, Polypeptide 3, 50KD (SNAPC3), TAF2D(TBP-associated factor), TAFII100(TBP-associated factor), TAFII130(TBP-associated factor), TAFII20(TBP-associated factor), TAFII250(TBP-associated factor), TAFII28(TBP-associated factor), TAFII30(TBP-associated factor), TAFII32(TBP-associated factor), TAFII40(TBP-associated factor), TAFII55(TBP-associated factor), TAFII80(TBP-associated factor), TBP(TATA Binding Protein), TMF1 (TATA Element Modulatory Factor 1), RPB 7.0, RPB 7.6, RPB 17, RPB 14.4, RNA polymerase I subunit hRPA39, 13.6 Kd Polypeptide (DNA-Directed RNA Polymerase II 13.6 kD Polypeptide), POLR2C(RNA polymerase II, polypeptide C (33kD)), Polypeptide A (220kd), RNA Polymerase II 23k, RNA polymerase II holoenzyme component (SRB7), RNA polymerase II subunit (hsRPB10), RNA polymerase II subunit (hsRPB8), RNA polymerase II subunit hsRPB4, RNA polymerase II subunit hsRPB7, RNA Polymerase II Subunit(DNA- Directed RNA Polymerases I, II, and III 7.3 kD polypeptide), TCEB1L(Transcription elongation factor B (SIII), polypeptide 1-like), RNA polymerase III subunit (RPC39), RNA polymerase III subunit (RPC62), Elongation Factor 1-Beta, Elongation Factor S-II, TCEA (110kD), TCEB1, TCEB (18kD), TCEB1L, TCEB3, TCEC (15kDa), TFIIS (Transcription

Elongation Factor IIS), E2F1 (E2F Transcription Factor), TFAP2A (Transcription Factor A2 Alpha), TFCP2 (Transcription Factor CP2), TFC12 (Transcription Factor 12), PRKDC (Protein Kinase, DNA activated catalytic subunit), SUPT6H, TFIIA gamma subunit, TFIIA delta, TFIIB related factor hBRF (HBRF), TFIIE Alpha Subunit, TFIIE Beta Subunit, TFIIF, Beta Subunit, GTF2F1 (TFIIF), GTF2F2 (TFIIF), General Transcription Factor IIIA, TFIIH(52 kD subunit of transcription factor), TFIIH(p89), TFIIH(p80), TFIIH(p62), TFIIH(p44), TFIIH(p34), Transcription Factor IIf(General transcription factor IIF, polypeptide 1 (74kD subunit))Transcription Factor IIf(General transcription factor IIF, polypeptide 1 (74kD subunit)), BTF 62 kDSubunit (Basic transcription factor 62 kD subunit), CAMP-dependent transcription factor ATF-4, CCAAT box-binding transcription factor 1, CRM1(Negative regulator CRM1), Cyclic-AMP-dependent transcription factor ATF-1, GABPA(GA-binding protein transcription factor, alpha subunit (60kD)), ISGF-3(Signal transducer and activator of transcription 1-alpha/beta), NFIX(Nuclear factor I/X (CCAAT-binding transcription factor)), NFYA(Nuclear transcription factor Y, alpha), NTF97(Nuclear factor p97), Nuclear factor I-B2 (NFIB2), Nuclear factor NF45, Nuclear factor NF90, POU2F1(POU domain, class 2, transcription factor 1), Sp2 transcription factor, TCF12(Transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)), TCF3(Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)), TCF6L1(Transcription factor 6-like 1), TF P65(Transcription factor p65), TFCOUP2(Transcription factor COUP 2 (a.k.a. ARP1)), Transcription factor IL-4 Stat, Transcription Factor S-II (Transcription factor S-II-related protein), Transcription factor Stat5b, Transcription Factor, Transcription factor (CBFB), 9G8 Splicing Factor (Pre-mRNA Splicing factor SRP20), CC1.3(Splicing factor (CC1.3)), HnRNP F protein, HNRPA2B1(Heterogeneous nuclear ribonucleoproteins A2/B1), HNRPG(Heterogeneous nuclear ribonucleoprotein G), HNRPK(Heterogeneous nuclear ribonucleoprotein K), Pre-mRNA splicing factor helicase, Pre-mRNA splicing factor SF2, P33 subunit, Pre-mRNA splicing factor SRP20, Pre-mRNA

splicing factor SRP75, PRP4(Serine/threonine-protein kinase PRP4), PTB-Associated Splicing Factor, Ribonucleoprotein A', Ribonucleoprotein A1, Ribonucleoprotein C1/C2, RNP Protein, L (Heterogeneous nuclear ribonucleoprotein L), RNP-Specific C(U1 small nuclear ribonucleoprotein C), SAP 145(Spliceosome associated protein), SAP 61(Splicesomal protein), SC35(Splicing factor), SF3a120, SFRS2(Splicing factor, arginine/serine-rich 2), SFRS5(Splicing factor, arginine/serine-rich 5), SFRS7(Splicing factor, arginine/serine-rich 7), Small nuclear ribonucleoprotein SM D1, SnRNP core protein Sm D2, SnRNP core protein Sm D3, SNRP70(U1 snRNP 70K protein), SNRPB(Small nuclear ribonucleoprotein polypeptides B and B1), SNRPE(Small nuclear ribonucleoprotein polypeptide E), SNRPN(Small nuclear ribonucleoprotein polypeptide N), Splicing factor SF3a120, Splicing factor U2AF 35 kD subunit, Splicing factor U2AF 65 kD subunit, SRP30C(Pre-mRNA splicing factor SF2, p33 subunit), SRP55-2(Pre-mRNA splicing factor SRP75), Transcription factor BTEB, Transcription initiation factor TFIID 250 kD subunit, Cleavage and polyadenylation specificity factor, Cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kD, Cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kD, HNRNP Methyltransferase, PABPL1(Poly(A)-binding protein-like 1), Pap mRNA(Poly(A) Polymerase), RNA unwinding, RNA Helicase, GU Protein (ATP-Dependent RNA helicase dead), KIAA0224 Gene(Putative ATP-dependent RNA helicase), RNA Helicase A, RNA Helicase P110, and Ste13(Nuclear RNA Helicase).

114. The method, inhibitor, pharmaceutical composition, or nucleic acid probe of any of claims 6, 18, 26, 34, 47, 63, 92, and 106, wherein said gene is selected from the group consisting of AP47(Clathrin Coat Assembly AP47), AP50(Clathrin Coat Assembly Protein AP50), Cell Surface Protein (Clathrin Heavy Polypeptide-Like Protein), Cltb(Clathrin Light Chain B), Cltc (Clathrin Heavy Chain), Adenylate Cyclase, Adenylate Cyclase, Adenylate Cyclase, II, Adenylate Cyclase,IV, Complex I, MTND1 (Subunit ND1), MTND2 (Subunit ND2), MTND3 (Subunit ND3), MTND4 (Subunit ND4), MTND4L (Subunit ND4L), MTND5 (Subunit ND5),

MTND6 (Subunit ND6), Complex II, Complex III, Cytochrome b subunit, Complex IV, CO1 (Cytochrome c Oxidase Subunit I), CO2 (Cytochrome c Oxidase Subunit 2), CO3 (Cytochrome c Oxidase Subunit 3), Complex V, ATP Synthase Subunit ATPase 6, Kinesin Heavy Chain, Kinesin Light Chain, Syntaxin 1a, Syntaxin 1b, Syntaxin 3, Syntaxin 5a, Syntaxin 7, CANX (Calnexin), ER Lumen Protein 1, ER Lumen Protein 2, Ribophorin I, Ribophorin II, Signal recognition particle receptor, SRP Protein, TIM17 preprotein translocase, Golgin-245, TGN46 (Trans-Golgi Network Integral Membrane Protein TGN38 Precursor), Beta-Cop, Coatomer Beta' Subunit, Coatomer Delta Subunit, Gp36b Glycoprotein (Vesicular integral-membrane protein VIP36 precursor), Homologue of yeast sec7, Protein transport protein SEC13 (Chromosome 3p25), SEC14 (S. Cerevisiae), Synaptic vesicle membrane protein VAT-1, Synaptobrevin-3, Synaptotagmin I, Transmembrane(COP-coated vesicle membrane protein p24 precursor), Vacuolar-Type (Clathrin-coated vesicle/synaptic vesicle proton pump 116 kd subunit), 140 kD Nucleolar phosphoprotein, Autoantigen p542, Export protein Rae1 (RAE1), Heterogeneous nuclear ribonucleoprotein A1, Nuclear pore complex protein hnup153, Nuclear pore complex protein NUP214, Nuclear pore glycoprotein p62, Nuclear Transport Factor 2, Nucleoporin 98 (NUP98), NUP88, Ribonucleoprotein A, Ribonucleoprotein B", Karyopherin, Importin Alpha Subunit, TRN (Transportin), Actin, Beta-Centractin, Capping Protein Alpha, CFL1 (Cofilin, Non-Muscle Isoform), Desmin, Dystrophin, Gelsolin, hOGG1(Myosin Light Chain Kinase), IC Heavy Chain, Itga2 (Integrin, Alpha 2 (CD49B, alpha 2 Subunit of VLA-2 receptor)), Itga3 (Integrin Alpha-3 Precursor), Keratin 19, Keratin, Type II, Lamin A, LBR(Lamin B Receptor), Light Chain Alkali, MacMarcks mRNA, MAP1a (Microtubule-Associated Protein 1A), MAP2(Microtubule-Associated Protein 2), MEG1(Protein-Tyrosine Phosphatase MEG1), Microtubule-Associated Protein TAU, Suppressor Of Tubulin STU2, TUBg (Tubulin Gamma Chain), Tubulin Alpha-4 Chain, USH1b (Myosin II Heavy Chain), Villin, Villin 2 (Ezrin), Actin Depolymerizing, Capping (Actin Filament), MYH9(Myosin, Heavy Polypeptide 9, Non-Muscle), MYL5(Myosin Regulatory

Light Chain 2), Myosin Heavy Chain 95F, Myosin Heavy Chain IB, Myosin IB, Sh3p17(Myosin IC Heavy Chain), Sh3p18(Myosin IC Heavy Chain), KIAA0059(Dematin:Actin-Bundling Protein), TTN (Titin:Myosin Light Chain Kinase), ATP6c(Vacuolar H+ ATPase proton channel subunit), ATP6a1 (ATPase, H+ Transporting, Lysosomal (Vacuolar Proton Pump), Alpha Polypeptide, 70kD), ATP6b1(ATPase, H+ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD), ATP6d(ATPase, H+ transporting, lysosomal (vacuolar proton pump) 42kD), ATP6e(ATPase, H+ transporting, lysosomal (vacuolar proton pump) 31kD), ATPase, H+ transporting, lysosomal (vacuolar proton pump) 31kD, and Superoxide Dismutase.

115. A method for identifying an inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on a conditionally essential gene, and wherein said gene is subject to loss of heterozygosity in a cancer, said method comprising the steps of:

(a) determining at least two alleles of a said gene;

(b) testing a potential allele specific inhibitor to determine whether said potential allele specific inhibitor is active on at least one but less than all of said alleles;

wherein inhibition of expression of at least one but less than all of said alleles or reduction of the level of activity of a product of at least one but less than all of said alleles in the presence of said potential allele specific inhibitor is indicative that said potential allele specific inhibitor is a said inhibitor.

116. An inhibitor potentially useful for treatment of cancer, wherein said inhibitor is active on an allelic form of a conditionally essential gene, said gene has at least two alternative alleles in a population, and

wherein said inhibitor targets at least one but less than all of said alternative alleles.

117. A pharmaceutical composition, comprising

at least one allele specific inhibitor targeting at least one but less than all allelic forms of a conditionally essential gene in a population; and

a pharmaceutically acceptable carrier or excipient.

118. A method for producing an inhibitor potentially useful for cancer treatment, wherein said inhibitor is active on at least one but less than all alternative alleles of a conditionally essential gene having at least two alternative alleles, comprising the steps of:

(a) identifying a conditionally essential gene that has alternative allelic forms in a noncancerous cell, wherein one of said alternative allelic forms is deleted in a cancer cell;

(b) screening to identify an inhibitor which inhibits said at least one but less than all of said at least two alternative alleles; and

(c) synthesizing said inhibitor in an amount sufficient to produce a therapeutic effect when administered to a patient suffering from a cancer in whom cancerous cells have only an allele of said gene inhibited by said inhibitor and in whom normal cells are heterozygous for said gene and contain an allelic form not inhibited by said inhibitor.

119. A method for preventing the development of cancer in a patient having a precancerous condition, comprising the steps of:

a. subjecting cells of said precancerous condition to an altered condition such that a first conditionally essential becomes essential;

b. administering to said patient a therapeutic amount of a first allele specific inhibitor targeted to an allele of said first conditionally essential gene present in cells of said precancerous condition, wherein the normal somatic cells of said patient are heterozygous for said first gene, said inhibitor is active on at least one but less than all allelic forms of said gene present in a population and targets only one allelic form present in said normal somatic cells; and

wherein cells of said precancerous condition have undergone LOH of said first gene.

120. The method of claim 119, wherein the cells of said precancerous condition are not clonal from a single cell, further comprising the step of:

c. serially administering to said patient at least one additional allele specific inhibitor, wherein each of said at least one additional allele specific inhibitors targets a different allele of a conditionally essential gene or an essential gene than is targeted by said first allele specific inhibitor, wherein said different allele may be a different allele of said first gene or an allele of a different gene, and wherein said patient is heterozygous for each targeted gene and each targeted gene has undergone LOH in cells of said precancerous condition.

121. A method for treating a patient suffering from a cancer, wherein said patient is heterozygous for a conditionally essential gene, comprising the steps of:

a) subjecting cells of said cancer to altered conditions such that said gene is essential; and

administering a therapeutic amount of an allele specific inhibitor active on at least one but less than all allelic forms of said gene present in a population,

wherein said allele specific inhibitor inhibits only one allelic form of said gene present in said patient, and said only one allelic form of said gene is present in cancer cells in said patient.

122. The method of claim 121, further comprising the steps of:

(a) determining whether non-cancerous cells of said patient are heterozygous for a particular conditionally essential gene; or

(b) determining whether cancerous cells of said patient have only one allele of said particular gene; or

(c) both (a) and (b).

123. A method of inhibiting growth of a cell comprising the steps of:

a) subjecting said cell to conditions such that said gene is essential; and

b) administering at least one inhibitor active on an allele of said conditionally essential gene,

wherein said inhibitor is less active on at least one other allele of said gene.

124. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a conditionally essential gene, wherein said patient is suffering from a cancer, said method comprising the step of:

identifying a patient heterozygous for a said gene,

wherein if said patient is heterozygous for said gene, then said patient is a potential patient for said treatment.

125. The method of claim 124, further comprising the step of determining whether cancer cells in said patient contain only a single allele of said gene,

wherein if said cancer cells contain only a single allele of said gene, then said patient is a potential patient for said treatment.

126. A method of identifying a potential patient for treatment with an inhibitor active on at least one but less than all alleles of a conditionally essential gene, wherein said patient is suffering from a cancer, said method comprising the step of:

determining whether cancer cells in said patient have undergone LOH of a said gene,

wherein if said cells have undergone LOH of said gene, then said patient is a potential patient for said treatment.

126. A nucleic acid probe at least 12 nucleotides in length which is perfectly complementary to a portion of a first allelic form of a conditionally essential gene,

wherein said portion comprises a sequence variance site, and wherein

said probe hybridizes under stringent hybridization conditions to said portion and not to a corresponding portion of a second allelic form having at least one different nucleotide at said sequence variance site.

127. A method for selecting a patient for treatment with an antiproliferative treatment, comprising the steps of:

a) determining whether normal somatic cells in a potential patient are heterozygous for an essential or conditionally essential gene, wherein a first allelic form of said gene is more active than a second allelic form, and wherein a reduction in the activity of said gene in a cell increases the sensitivity of said cell to a said antiproliferative treatment; and

b) determining whether cancer cells of said patient have only said second allelic form of said gene,

wherein if said somatic cells are heterozygous and said cancer cells have only said second allelic form, it is indicative that said patient is suitable for treatment with said antiproliferative treatment.

128. A method for selecting an antiproliferative treatment for a patient suffering from a cancer, comprising the steps of:

a) determining whether normal somatic cells in a potential patient are heterozygous for an essential or conditionally essential gene which reduces the sensitivity of cells to an antiproliferative treatment, wherein a first allelic form of said gene is more active than a second allelic form, and wherein a reduction in the activity of said gene in a cell increases the sensitivity of said cell to a said antiproliferative treatment; and

b) determining whether cancer cells of said patient have only said second allelic form of said gene,

wherein if said somatic cells are heterozygous for said gene and said cancer cells have only said second allelic form, it is indicative that said antiproliferative

treatment is suitable for said patient.

129. The method of any of claims 115-129, wherein said gene is selected from the group consisting of:

galactose-1-phosphate uridyltransferase, galactose kinase, UDP galactose-4-epimerase, methionine synthase, asparagine synthase, glutamine synthetase, multidrug resistance gne/Pglycoprotein, multidrug resistance associated proteins 1-5, bleomycin hydrolase, dihydropyrimidine dehydrogenase, β-ureidopropoinase, β-alanine synthetase, cytidine deaminase, thiopurine methyltransferase, CYP1A1, CYP1A2, CYP2A6, CYP2A7, CYP2B6, CYP2B7, CYP2C8, CYP2C9, CYP2C17, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP3A3, CYP3A4, CYP3A5, CYP3A7, CYP4B1, CYP7, CYP11, CYP17, CYP19, CYP21, CYP27, glutathione transferase alpha, glutathione transferase theta, glutathione transferae mu, glutathione transferase pi, methylguanine methyltransferase, 3-alkylguanine alkyltransferase, 3-methyladenine DNA glucosylase, DNA dependent protein kinase, catalytic subunit of DNA-PK, DNA binding subunit of DNA-PK Ku-70 or Ku-80 subunit, KARP-1, Poly(ADP-ribose) polymerase, Fanconi Anemia genes A, B, C, D, E, F, G, and H, ERCC-1, ERCC2/XPD, ERCC3/XPB, ERCC4, ERCC5, ERCC6, XPA, XPC, XPE, HHR23A, HHR23B, uracil glycosylase, 3-methyl adenine DNA glycosylase, NF-kappa B, XRCC4, XRCC5/Ku80, XRCC6, XRCC7, glutathione-X-transferase, I-kappa B alpha, HSP70, HSP27, and 9-oxoguanine DNA glycosylase.

131. A method for identifying a potential patient undergoing transplantation for treatment with an inhibitor active on at least one but less than all alleles of an essential gene, comprising the step of:

identifying a patient undergoing an allogenic bone marrow transplantation in which the donor tissue contains at least one alternative allele of an essential gene different from the alleles in somatic cells of said patient.

132. The method of claim 131, wherein said donor or said recipient is homozygous for an alternative allelic form of an essential gene that is not present in the other of said donor or said recipient.

133. A method for treating graft versus host disease in a patient receiving allogenic bone marrow transplantation, said method comprising the step of

administering to said patient at least one allele specific inhibitor specific for at least one but less than all of the allelic forms of an essential gene in a population, wherein said inhibitor inhibits stimulation of the donor immune system, and cells of the said patient comprise an allelic form of said gene not present in the donor bone marrow.

134. The method of claim 133, wherein said allele specific inhibitor is selected by identifying at least one alternative alleles of an essential gene present in the donor tissues but absent in the normal somatic cells of said patient; and

selecting a said inhibitor active on a said alternative allele of an essential gene present in said donor tissues but absent in the normal somatic cells of said patient.

135. The method of claim 134, wherein said at least one inhibitor recognizes both alleles of said essential gene that are present in said donor, but not both alleles of said gene that are present in said patient.

136. A method for enhancing engraftment of an allogenic bone marrow transplant, comprising the step of administering to a patient receiving said transplant an allele specific inhibitor which kills or suppresses the patient's bone marrow but not the donor bone marrow, thereby providing space for engraftment of the donor cells within the marrow cavity.

137. The method of claim 136, wherein the allele specific inhibitor is selected by

identifying alternative alleles of an essential gene that are present in the recipient but not the donor marrow.

138. The method of claim 137, wherein said allele specific inhibitor recognizes both allelic forms of the essential gene that are present in the recipient, but not both allelic forms of the same gene that are present in the recipient.

139. A method for treating or preventing chimerism in allogenic bone marrow transplantation, comprising

selectively killing or suppressing proliferation of the patient's own cells without toxicity to the donor cells by

administering to a patient receiving said transplantation at least one allele specific inhibitor active on at least one but less than all alternative alleles of a gene vital for cell growth or viability, wherein said inhibitor targets the allelic form or forms of a gene in bone marrow of said patient but does not target at least one allelic form of said gene in the donor bone marrow.

140. A method for treating cancer in a patient receiving allogenic or autologous transplantation, comprising the step of

administering to said patient at least one allele specific inhibitor which kills or inhibits the growth of cancer cells without toxicity to the transplanted marrow.

141. The method of claim 141, wherein said transplantation is autologous transplantation and said at least one allele specific inhibitor is selected to be active on the allele of an essential gene remaining in the cancer cells due to LOH in patients whose normal somatic cells are heterozygous for said essential gene, but not on the alternative allele of said gene present in said normal somatic cells,

whereby said administration enables continuing therapy of cancer without suppression of the transplanted marrow.

142. The method of claim 140, wherein said transplantation is allogenic transplantation and said allele specific inhibitor recognizes both alleles of said essential gene that are present in the recipient, but not both forms of the said gene that are present in said patient.

143. A method for eliminating malignant cells from transplanted marrow during autologous transplantation of a patient heterozygous for an essential gene, comprising

contacting cells from harvested autologous bone marrow *ex vivo* with at least one allele specific inhibitor active on at least one but less than all alternative alleles of said essential gene, wherein said inhibitor targets an allelic form of said gene present in cancer cells of said patient but does not target an alternative allele of said gene present in normal cells from said autologous bone marrow,

wherein said gene has undergone LOH in cancer cells of said patient.

144. The method of claim 143, wherein said autologous bone marrow is harvested from said patient prior to high dose radiation or chemotherapy.

145. The method of claim 143, further comprising the steps of:

a. identifying one alternative allele of an essential gene remaining in the cancer cell due to LOH in patients who are heterologous with two different alternative forms of the essential gene in normal cells of the autologous bone marrow;

b. cultivating said autologous bone marrow *ex vivo* in the presence of an allele specific inhibitor that inhibits the allele that is present in the cancer cells, but not the heterologous allele that is present in the normal bone marrow. .

146. The method of claim 143, wherein said autologous bone marrow is contacted with a plurality of said allele specific inhibitors.

147. A method for separating a first cell from a mixture of cells, comprising the steps of:

a) providing an allele specific binding compound which binds to at least one but less than all alleles of a gene, wherein a said allele of said gene expressed in said first cell is not expressed in other cells of said mixure of cells or is expressed in other cells in said mixture of cells and not in said first cell;

b) contacting said mixture of cells with said binding compound under conditions such that said binding compound binds to said allele and not to non-target alleles; and

c) separating bound cells from unbound cells.

148. The method of claim 147, wherein said mixture of cells comprises normal somatic cells and cancer cells from a patient, said first cells are said normal somatic cells, and said first cells express a said allele deleted in said cancer cells due to LOH of said gene, comprising

separating said normal somatic cells from said cancer cells.

149. The method of claim 147, wherein said allele specific binding compound is an antibody or antibody fragment.

150. The method of claim 147, wherein said binding compound is attached to a solid support.

Fig. 1

Target Gene Summary Table
Dihydropyrimidine Dehydrogenase
Chromosome 1p22-1q21
VARIA950

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Protein	Race Specific heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
DPD1a-DPD2b	bp166	T,C	TT	TT	TT	CC	TT	TC	CC	TT	TC	TC	TC	TT	TT	TT	TT	TT	TT		TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	11%		Cys/Arg	
DPD1c-DPD2a	bp 577	A,G	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	9%		Met/Val	
DPD9a-DPD10b	bp 3925	G,A		GG	AG	AA	AA	AG	AA		AG	AA	AG	AG	AA	AA	AA	AA	AA			AA	AA	GG	AG	AG	GG	AA	AA	AG	AG	AG	GG	AA	AA	GG		AG	35%	These are separated by 11 bases		50 % in blacks and chinese
DPD9a-DPD10b	bp 3937	C,T	CC	CC	CC	TC	TT	TC	TC	CC	CC	CC	CC	TC	TC	TC	TT	CC	CC	TC	CC	TT		CC	TC	TC		TC	TT		TC	CC		CC	TT	CC	TT	TC	38%			
			w	b	b	b	a	w,a	b	w	ash	h	ar,p	h	j	c	a	j	c	c	a	f	f	w	j	pr	w	w	w	w	w	w	w	w	w	w	w	wh	56% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA500.2.1	166 T	TGCAACTCTGTGTTCCACTTC
VARIA500.2.2	166 C	TGCAACTCTGCGTTCCACTTC
VARIA500.3.1	577 A	ATTCAAAGCAATGAGTATCCC
VARIA500.3.2	577 G	ATTCAAAGCAGTGAGTATCCC
VARIA500.1.1	3925 G	CCCACTCTTTTGCTGTGCACAT
VARIA500.1.2	3925 A	CCCACTCTTTTACTGTGCACAT
VARIA500.4.1	3937 C	TGTGCACATACGGGCTCTGAC
VARIA500.4.2	3937 T	TGTGCACATATGGGCTCTGAC

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence nomenclature and numbering from:
Yokota,H., Fernandez-Salguero,P., Furuya,H., Lin,K., McBride,O.W., Podschun,B., Schnackertz,K.D., and Gonzalez, F.J. "cDNA Cloning and Chromosome Mapping of Human Dihydropyrimidine Dehydrogenase, an Enzyme Associated With 5-Fluorouracil Toxicity and Congenital Thymine Uraciluria." Journal of Biological Chemistry, 269 (37) 23192-23196 (1994)

Validation Status:

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

10/30/96

WO 98/41648 PCT/US98/05419

Target Gene Summary Table
Thymidylate Synthase
Chromosome 18p11.32
VARIA250

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Protein	Race Specific Heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
TS3b-TS4a	1140	C,T	TC	TC	TT	TC	TT	CC	TC	TT	CC	CC	TC	CC	TT	TT	TC	TC	TC	TC	TC	TT	TC	TC	TC	CC	CC	TC	TC	TT	CC	CC	CC	TT	TC	TC	TC	TC	53%		3'UT	wide distribution
TS3b-TS4a	1210	A,G	AG	AG	AA	AG	AG	AA	AA	GG	AA	AA	AG	AA	AG	AA	AA	AA	AG	AG	AA	GG	AG	AG	AG	AA	AA	AG	AG	GG	AA	AA	AA	GG	AA	AG	AA	AG	42%		3'UT	wide distribution
TS3o-rp3	1571	A,T	AT	AT	TT	AT	TT	AA	AT	TT	AA	AA	AT	AA	TT	TT	AT	AT	AT	AT	AT	TT	AT	AT	AT	AA	AA	AT	AT	TT	AA	AA	AA	TT	AT	AT	AT	AT	53%		3'UT	wide distribution
	276	T,C																																							tyr33his	Probably rare
			w	b	b	b	a	w/a	b	w	ash	h	mu	h	j	g	c	j	c	c	a	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	wR	58%	= Locus Heterozygosity		

ID#	Allele	Sequence around polymorphism*
VARIA250.1.1	1140 C	CAAAGGAGCTCGAAGGATATT
VARIA250.1.2	1140 T	CAAAGGAGCTTGAAGGATATT
VARIA250.2.1	1210 A	TCTAAAAGAAAAAGGAACTAG
VARIA250.2.2	1210 G	TCTAAAAGAAGAAGGAACTAG
VARIA250.3.1	1571 A	ATGAACTTTAAAGTTATAGTT
VARIA250.3.2	1571 T	ATGAACTTTATAGTTATAGTT
VARIA250.4.1	276 T	GGAGCTGCAGTACCTGGGGCA
VARIA250.4.2	276 C	GGAGCTGCAGCACCTGGGGCA

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms:

#	%
1	3

Sequence from:
GenBank accession # D00596 (This accession has the genomic sequence, including intron/exon boundaries.)
Kaneda, S., Takeishi, K., Shimizu, K., Gotoh, O. and T. Seno, Ayusawa, D., (1990) *Structural and Functional Analysis of the Human Thymidylate Synthase Gene. J. Biological Chemistry* 265: 20277-20284.

Validation:
The cytotoxic drug 5-fluorouracil acts by specifically inhibiting thymidylate synthase.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped
None

Target Gene Summary Table
Threonyl-tRNA Synthetase
Chromosome 5p13-cen
VARIA302

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																Het%	Comments	Protein	Race Specific Heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
Thr3b-Thr4	bp 1608	G,A	GG							AG																	AG	GG	GG	AG	GG	GG	GG	GG	AG	GG	GG	AG	45%	20 caucasians tested	silent	9/20 = 45% in Caucasians
THRS-THR4	bp1755	A,G	GG	GG	GG	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GA	GA	GG	GG	GG	GG	GG	GG	GA	GA	GG	GA	GA	GA	GG	GA	GA	GA	GG	GG	GG	GA	GG	31%		silent	40% in Caucasians 50% in Chinese
TARS5d-8a	bp 2395	C,T	TT	TT	TC	TT	TT	TT	CC	TT	TT	TT	TT	TT	TT	TC	TC	TT	CC	TT	TT	TT	TT	TT	TT	TC	TT	TC	TC	TT	TC	TT	TC	TT	TT	TT	TC	TT	25%		3' UT	30% in Caucasians 50% in Chinese
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c	a	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w	w,it	47%	= Locus Heterozygosity		

ID#	Allele	Sequence around polymorphism*
VARIA302.1.1	1608 G	CTACTCGCCCGGAAAAATTCC
VARIA302.1.2	1608 A	CTACTCGCCCAGAAAAATTCC
VARIA302.2.1	1755 G	TTAAAGATGCGATTGGGCGGT
VARIA302.2.2	1755 A	TTAAAGATGCAATTGGGCGGT
VARIA302.3.1	2395 T	TGGCAAAGTCTGAAATAGGTC
VARIA302.3.2	2395 C	TGGCAAAGTCCGAAATAGGTC

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms:

#	%
2	3%

Sequence from:
GenBank accession # M63180
Cruzen,M.E. & S.M. Arfin (1991) Nucleotide and deduced amino acid sequence of human threonyl-tRNA synthetase reveals extensive homology to the Escherichia coli and yeast enzymes. *J. Biol. Chem.* 266: 9919-9923.

Validation:
Multiple other tRNA synthetases have been proven essential for cell survival.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
none

Target Gene Summary Table
TATA Associated Factor 2H
Chromosome 11p15.2-15.5
VARIA520

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Location	Race Specific heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
TAF8-TAF2	554	G,A	GG	GG	GA	GG	GG	GG	GA	GG	GG	GA	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	AA	GA	GA	GG	GG	GG	GG	GG	GG	GG	GG	GA	GA	AA	22%			50% of Blacks, all Hispanics
			w	b	b	b	a	w,g	b	w	ash	h	w,g	h	j	c	c	j	c	c	a	j	j	w	i	pr	w	w	w	w	w	w	w	w	w	w	wh	22%=Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA520.1.1	554	TGAAGGGCACGGCCTCCGGCA
VARIA520.1.2	554	TGAAGGGCACAGCCTCCGGCA

*Bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession # U13991
Jacq, X., Brou, C., Lutz, Y., Davidson, I., Chambon, P. and L. Tora (1994) Human TAFII30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell* 79: 107-117. (Note: the numbering in the GenBank accession and the Cell 79:107-117 paper differ by two nucleotides; the polymorphism is at nucleotide 556 using the numbering in the paper.)

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Validation:
Other TATA associated factors (TAFs) have been proven essential for cell growth.

Other populations genotyped:

Target Gene Summary Table
Ribonucleotide Reductase, M1 Subunit
Chromosome 11p15.5
VARIA200

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																		Het%	Comments	Location	Race Specific Heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
RR1e-RR2	bp 1037	C,A	CC	CC	CC	CC	AC	AA	CC	AA	AC	AC	CC	AC	CC	CC	CC	CC	CC	AC	CC	AC	CC	AC	AC		AC			AC	AC	AA	CC	AA	CC	AA	CC		33%		silent	
RR5b-RR6c	bp 2410	A,G	AA	AA	AG	AG	AG	GG	AA	GG	AG	AG	AA	GG	AA		AA	AA	AA	AG	AA	GG	AA	AG	AG	AG	AG	AA	AA	AG	AG	GG	AA	GG	AA	GG	AG	AG	40%	These two polymorphisms are separated by 8 bases	silent	50% in Blacks
RR5b-RR6c	bp2419	A,G	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AG		AG	AA	AG	AA	GG	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	20%		silent	50% in Asians
RR5c-RR6b	bp2717	T,A	TT	TT	TT	TT	TT	TT	AT	TT	TT	TT	TT	AT	AT	TT	AT	TT	AT	TT	AA	AT	AT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	19%	These two polymorphisms are separated by 5 bases	3' UT	50% in Asians
RR5c-RR6b	bp 2724	T9, T10								T9 T9									T9 T10		T10 T10					T9 T9														3' UT	
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c	a	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w		58% = Locus Heterozygosity		

ID number	Allele	Sequence around polymorphism*
VARIA200.1.1	1037 C	CAACACAGCTCGATATGTGGA
VARIA200.1.2	1037 A	CAACACAGCTAGATATGTGGA
VARIA200.2.1	2410 A	ATTTAAGGACAAGACCAGCAG
VARIA200.2.2	2410 G	ATTTAAGGACGAGACCAGCAG
VARIA200.3.1	2419 A	CAAGACCAGCAGCTAATCCAA
VARIA200.3.2	2419 G	CAAGACCAGCGGCTAATCCAA
VARIA200.4.1	2717 T	GTTAATGATGTTAATGATTTT
VARIA200.4.2	2717 A	GTTAATGATGATAATGATTTT
VARIA200.5.1	2724 T9	ATGATAATGA(T)9 AAACTCATAT+
VARIA200.5.2	2724 T10	ATGATAATGA(T)10AAACTCATAT+

*bold nucleotide is the polymorphic base

+Number after parends indicates length of homopolymeric repeat

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession # X59543

Parker,N.J., Begley,C.G. and R.M. Fox (1991) Human M1 Subunit of Riobonucleotide Reductase: cDNA Sequence and Expression in Stimulated Lymphocytes. *Nucleic Acids Res.* 19: 3741-3741.

Validation:
Hydroxyurea is a cytotoxic drug which specifically binds and inhibits ribonucleotide reductase.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

WO 98/41648 PCT/US98/05419

Target Gene Summary Table
Ribosomal Protein S14
Chromosome 5q23-q33
VARIA326

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																Het%	Comments	Protein changes	Race Specific heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
rpS1-rpS2	183	A,G	GG	GG	GG	AG	AG	GG	AG	AG	GG	GG	GG	GG	AG	AG	AG	GG	GG	GG	GG	AG	GG	GG	GG	AG	GG	AG	AG	AG	GG	GG	GG	AG	GG	AG	AG	AG	44%		Silent	50% in Whites
			w	b	b	b	a	w/i	b	w	ash	h	cu/i	h	j	c	c	j	c	c	a	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w/i	44 % = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA326.1.1	183 G	TTTCTGGCAAGGAAACCATCT
VARIA326.1.2	183 A	TTTCTGGCAAAGAAACCATCT

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession #'s M13934 &M13641 (genomic and cDNA sequences)

Rhoads, D.D., Dixit,A. and D.J. Roufa (1986) Primary Structure of Human Ribosomal Protein S14 and the Gene That Encodes It. *Molecular and Cellular Biology* 6: 2774-2783.

Validation:
The poison emetine inhibits ribosome function by specifically interacting with RPS14.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

Target Gene Summary Table
Replication Protein A, 70 kDa Subunit
Chromosome 17p13.3
VARIA401

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals	Het%	Comments	Protein	Race Specific Heterozygosity
RPA70.1-RPA70.2e	81	G,A	GG AA GG GG AA GG AG GG AA AG AG GG AA GG AA AG AA GG AA AA GG AG AG GG GG GG AG GG GG GG AG GG AG	25%	within 8 bases of ATG	silent	31% in Caucasians
RPA701d-RP704f	1120	A,G	AG AA AA AA AA AA AA AG AG AA AA AG AA AA AA AA AA AA AA AA AA AA AA AA AA AA AA AA AG AG AG AG AA AA	25%		ala351thr	44% in Caucasians
RPA701d-RP704f	1124	C, T			rare	ser352phe	
RPA701d-RP704f	1125	C, T				silent	21% in Swedes
RP703aa-RP704c	1674	T,C	TT CT CT TT TT CT TT TT TT TT TT TT CT TT CT TT CT TT TT CT TT TT TT CT CT TT TT T1 T1 CT T1 TT CT TT TT	31%		silent	50% in Blacks 44% in Asians
RP703c-RP704a	2046	T2, T3	3,3 3,3 3,3 3,3		within 4 bases of 2050	3'UT	
RP703c-RP704a	2050	T,C	CT CT CT CT CT CC CC CT TT CC CC CT CC CC CC CC CC CT CT CC CC CC CC CC CT CC CT CC CT CC CT CC CT	42%	within 4 bases of 2046	3'UT	75% in Blacks 50% in Caucasians
RP703e-rRP70	2297	C8,C9	8,8 8,8 8,8 8,8 8,8 9,9 9,9 8,8 8,8 9,9 9,9 9,9 8,8 9,9 9,9 9,9 9,9 9,9 8,8 8,8 9,9 9,9 9,9 9,9 9,9 8,8 8,8 9,9 9,9 8,8 8,8 9,9 9,9 8,8	33%		3'UT	75% in Blacks 50% in Caucasians
RP703e-rRP70	2341	A, G	GG GG AA GG			3'UT	
			w b b b a w g b w ash h cu g h j c c j c c a j j ar i pr w w w w w w w w w w w w	78% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA401.1.1	81 G	*ATG* GTCGGCCAGCTGAGCGAGG
VARIA401.1.2	81 A	*ATG* GTCGGCCAACTGAGCGAGG
VARIA401.2.1	1120 A	CTTGATGGACACATCCGGGAA
VARIA401.2.2	1120 G	CTTGATGGACGCATCCGGGAA
VARIA401.3.1	1674 T	TCCAGGAGTCTGCTGAAGCTA
VARIA401.3.2	1674 C	TCCAGGAGTCCGCTGAAGCTA
VARIA401.4.1	2046 T2	GACTAAGCAA (T)2 CCTCCCTCGT+
VARIA401.4.2	2046 T3	GACTAAGCAA (T)3 CCTCCCTCGT+
VARIA401.5.1	2050 T	AAGCAATTCCTCCCTCGTGCG
VARIA401.5.2	2050 C	AAGCAATTCCCCCCTCGTGCG
VARIA401.6.1	2297 C8	GTGGTGACCA (C)8 ATCCCCGCTC
VARIA401.6.2	2297 C9	GTGGTGACCA (C)9 ATCCCCGCTC
VARIA401.7.1	2341 A	TCAGCGGGGCAAGCTGAGAAG+
VARIA401.7.2	2341 G	TCAGCGGGGCGAGCTGAGAAG+

* bold nucleotide is the polymorphic base

\+ The number after the parends indicates the length of the homopolymeric segment.

Other SSCP Polymorphisms: ID# %

Sequence from:
GenBank accession # M63488

Erdile,L.F., Heyer,W.D., Kolodner,R. and T.J. Kelly (1991) Characterization of a cDNA encoding the 70-kDa single-stranded DNA-binding subunit of human replication protein A and the role of the protein in DNA replication. *J. Biol. Chem.* 266: 12090-12098.

Validation:
RPA has been proven essential for yeast viability and for mammalian DNA replication *in vitro* .

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
nt 1124 - very rare allele in Swedes
nt 1125 - 21% heterozygous in Swedes

Target Gene Summary Table
Replication Protein A, 32 kDa Subunit
Chromosome 1p35
VARIA402

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Location	Race Specific Heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
RPA32 1-RPA32 2	bp 40	G,A	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GG	GA	GG	GG	GG	GA	GG	GA	GG	GG	GG	11%		5'UT	25% in Caucasians
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	a	j	c	c	a	j	j	w	i	pr	w	w	w	w	w	w	w	w	w	w	w,i	11% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA402.1.1	40 G	CCCCAGACCC**G**CACCTTCTCG
VARIA402.1.2	40 A	CCCCAGACCC**A**CACCTTCTCG

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession # J05249

Erdile, L.F., Wold, M.S. & T.J. Kelly (1990). The primary structure of the 32-kDa subunit of human replication protein A. *J. Biol. Chem.* 265: 3177-3182.

Validation:
RPA has been proven essential for mammalian DNA replication *in vitro* and for yeast viability.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

Target Gene Summary Table
RNA Polymerase II, 220 kDa Subunit
Chromosome 17p13
VARIA500

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																		Het%	Comments	Protein changes	Race Specific Heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
POL2 F3-R3	857	G,A	GG	GG	GA	GG	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GA	GA	GG	GG	GA	GG	GA	GG	GA	GG	GG	GG	GG	GG	22%		silent	38% in Caucasians
POL2 F5-R5	1260	C,T	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	CT	cc	cc	cc	cc	cc	CT	cc	cc	cc	6%		arg292cys	13% in Caucasians
POL2 F5-R5	1346	C,T	cc	cc	cc	CT	cc	cc	cc	cc	cc	CT	cc	CT	cc	cc	CT	CT	cc	CT	cc	cc	cc	TT	cc	cc	cc	cc	cc	CT	CT	CT	CT	CT	CT	cc	CT	CT	39%		silent	50% in Caucasians
POL2 F6-R6	1544	C,T	cc	cc	CT	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	3%		silent	
POL2 F7-R7	1847	C,T	cc	cc	CT	CT	cc	cc	cc	CT	cc	cc	cc	cc	cc	cc	CT	CT	cc	cc	cc	cc	cc	cc	CT	CT	CT	cc	cc	CT	cc	CT	cc	CT	cc	cc	cc	cc	31%		silent	50% in Blacks
POL2 F12-R12	2678	C,T	cc	cc	cc	CT	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	CT	CT	cc	CT	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	11%		silent	50% in Chinese
POL2 F13-R13	3059	C,T	cc	CT	cc	cc	CT	TT	CT	CT	cc	cc	cc	cc	cc	CT	cc	cc	CT	CT	cc	cc	cc	cc	CT	cc	cc	CT	cc	cc	cc	cc	CT	cc	cc	CT	cc	cc	31%		silent	75% in Chinese
POL2 F17-R17	3827	C,T	cc	cc	CT	cc	cc	cc	cc	CT	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	CT	TT	CT	cc	cc	CT	cc	CT	cc	CT	cc	cc	cc	cc	22%		silent	38% in Caucasian
POL2 F30-R30	6466	T,C	TT	TC	TC	TC	cc	cc	cc	TT	TT	TT	TT	TT	TC	TC	TC	TC	TC	cc	TC	TT	TC	TT	TT	TC	TT	TC	TT	TC	TT	TC	TC	TC	TT	TC	TT	TT	60%		3' UT	70% Asian,75% Black
POL2 F30-R30	6557	T,C	TT	TC	TT	TC	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	6%		3' UT	
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c	a	j	j	ar	j	pr	w	w	w	w	w	w	w	w	w	w	w,it	83% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism
VARIA500.1.1	857 G	GCGAGGGTGGGGAGGAGATGG
VARIA500.1.2	857 A	GCGAGGGTGGAGAGGAGATGG
VARIA500.2.1	1260 C	TCAGCTGCGGCGCAATGAGCA
VARIA500.2.2	1260 T	TCAGCTGCGGTGCAATGAGCA
VARIA500.3.1	1346 C	TGGTGGACAACGAGCTGCCTG
VARIA500.3.2	1346 T	TGGTGGACAATGAGCTGCCTG
VARIA500.4.1	1544 C	CCATTGCTGCCAACATGACCT
VARIA500.4.2	1544 T	CCATTGCTGCTAACATGACCT
VARIA500.5.1	1847 C	TGAATCTTAGCGTGACAACTC
VARIA500.5.2	1847 T	TGAATCTTAGTGTGACAACTC
VARIA500.6.1	2678 C	CTGAATACAACAACTTCAAGT
VARIA500.6.2	2678 T	CTGAATACAATAACTTCAAGT
VARIA500.7.1	3059 C	AGCTGCGCTACGGCGAAGACG
VARIA500.7.2	3059 T	AGCTGCGCTATGGCGAAGACG
VARIA500.8.1	3827 C	TGGGCCAGTCCGCTCGAGATG
VARIA500.8.2	3827 T	TGGGCCAGTCTGCTCGAGATG
VARIA500.9.1	6466 T	CTGATGCAGATTCTTGTCTTG
VARIA500.9.2	6466 C	CTGATGCAGACTCTTGTCTTG
VARIA500.10.1	6557 T	TGTCCCCAAATTGAAGATCCT
VARIA500.10.2	6557 C	TGTCCCCAAACTGAAGATCCT

* bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:

GenBank accession # X63564

Wintzerith,M., Acker,J., Vicaire,S., Vigneron,M. and C. Kedinger (1992) Complete Sequence of the Human RNA Polymerase II Largest Subunit. *Nucleic Acids Research* 20: 910.

Validation:

α-amanitin is a potent cytotoxin which specifically binds the 220 kDa subunit and inhibits RNA Polymerase II.

Ethnic & Racial Groups Surveyed:

a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:

None

Target G ne Summary Table
Glutaminyl-tRNA Synthetase
Chromosome 3p21
VARIA305

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Protein change	Race specific heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
Gln1-Gln2	*404*	C,T	CT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CC	CC	CC	CC	CC	CC	CT	CT	CC	CC	CC	CC	CC	CC	CC	11%			*19% in Whites*
			w	b	b	b	a	w,g	b	w	ash	h	cu,p	h	j	c	a	j	c	c	s	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w	w,g	11% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA305.1.1	404 C	TTAACAGGCA**C**CGGCCCCAGC
VARIA305.1.2	404 T	TTAACAGGCA**T**CGGCCCCAGC

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms:

#	%
1	3%
1	6%
2	8%

Sequence from:
GenBank accession # X76013
Lamour,V., Quevillon,S., Diriong,S., N'Guyen,V.C., Lipinski,M., and M. Mirande (1994) Evolution of the Glx-tRNA synthetase family: The glutaminyl enzyme as a case of horizontal gene transfer. *Proc. Natl. Acad. Sci. USA* 91: 8670-8674.

Validation:
Multiple tRNA synthetases have been proven essential for cell survival.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

3/4/98

WO 98/41648 PCT/US98/05419

Target Gene Summary Table
Sodium, Potassium ATPase, α1 Subunit
Chromosome 1p13-p11
VARIA125

Primer Pair	Location	Base	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			Genotypes of 36 unrelated individuals																	
F21-R19	1059	A,C	AA	AC	CC	AA	AA	AA	AC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
F10-R20	1428	G,A	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
F27-R28	2538	T,C	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
F16-R12	3324	C,T	CC	CC	CT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
F28-R27	3375	G,A	GG	GG	GA	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
F28-R27	3397	G,A	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
F28-R27	3408	C,A	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c

Primer Pair	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Het%	Comments	Protein Change	Race Specific Heterozygosity
F21-R19	AA	AA	AA	AA	AC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	11%		silent	50% of Blacks
F10-R20	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	6%		silent	
F27-R28	TT	TT	TT	TT	TT	TT	TT	TT	TC	TT	TT	TC	TT	TT	TC	TT	TC	TT	11%		Asp740Glu	25% of Whites
F16-R12	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	3%		silent	
F28-R27	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	6%		silent	50% of Blacks
F28-R27	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	3%		3' UTR	
F28-R27	CC	CC	CC	CC	CC	CC	CC	CC	CC	CA	CC	CC	CC	CC	CC	CC	CC	CC	3%		3' UTR	
	a	i	i	ar	it	pr	w	w	w	w	w	w	w	w	w	w	w	wh	28%	= Locus Heterozygosity		

ID#	Allele	Sequence around polymorphism
VARIA 125.1.1	1059	TCTTTTCAACAAATTGTGTTG
VARIA 125.1.2	1059	TCTTTTCAACCAATTGTGTTG
VARIA 125.2.1	1428	TGGGGTCCACGTCCACCATCT
VARIA 125.2.2	1428	TGGGGTCCACATCCACCATCT
VARIA 125.3.1	2538	CTGGCTCAGATGTGTCCAAGC
VARIA 125.3.2	2538	CTGGCTCAGACGTGTCCAAGC
VARIA 125.4.1	3324	TCGTATATGACGAAGTCAGAA
VARIA 125.4.2	3324	TCGTATATGATGAAGTCAGAA
VARIA 125.5.1	3375	GGGTGGAGAAGGAAACCTACT
VARIA 125.5.2	3375	GGGTGGAGAAAGAAACCTACT
VARIA 125.6.1	3397	TTAGCCCCCGTCCTGCACGC
VARIA 125.6.2	3397	TTAGCCCCCATCCTGCACGC
VARIA 125.7.1	3408	TCCTGCACGCCGTGGAGCATC
VARIA 125.7.2	3408	TCCTGCACGCAGTGGAGCATC
		*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank Accession #: D00099

Kawakami, K., Ohta, T., Nojima, H., and K. Nagano (1986). Primary structure of the alpha-subunit of human Na,K-ATPase deduced from cDNA sequence. *J. Biochem.* 100: 389-397.

Validation:
Ouabain is a potent cytotoxic drug which inhibits Na, K-ATPase by interactions with the α1 subunit.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:

Target Gene Summary Table
Lysyl-tRNA Synthetase
Chromosome 16q23-24
VARIA303

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Protein changes	Race Specific Patterns
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
Lys1-Lys2	89	A,G	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AG	AA	AG	AG	AG	AA	19%			31% in Whites
Lys3-Lys4	1798	G,C	cc	cc	gc	gc	cc	cc	gg	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	6%		ser,thr	50% in Blacks
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c	e	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w	w	25% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA303.1.1	89 A	AGCTGAAGAGACGCCTGAAAG
VARIA303.1.2	89 G	AGCTGAAGAGGCGCCTGAAAG
VARIA303.2.1	1789 G	ACAGTTGGCAGTTCTGTCTAG
VARIA303.2.2	1789 C	ACAGTTGGCACTTCTGTCTAG

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms:

#	%
2	3%

Sequence from:
GenBank accession # D31890

Nomura,N., Miyajima,N., Sazuka,T., Tanaka,A., Kawarabayashi,Y., Nagase,T., Ishikawa,K., Seki,T. & S. Tabata (1993) Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 35 genes deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG1. Unpublished

Validation:
Multiple other tRNA synthetases have been proven essential for cell survival.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

Target Gene Summary Table
Glutamyl Prolyl-tRNA Synthetase
Chromosome 1q32-q42
VARIA300

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Het%	Comments	Protein changes	Race Specific Heterozygosity
QP5a-QP6d	2520	C,A	CC	CA	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CA	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	7%	3/40 total	pro821his	25% in Blacks
QP5-QP6b	2944	G,A	AG	GG																																			13%	8448 total (48 are tumor lines)	silent	
QP5f-QP6b	2963	C,T																																						1/12 total	his969tyr	
QP5f-QP6b	2969	A,G	AA	AG	AA	AA	AA	AG	AA	AA	AG	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AG	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	24%	10/42 total	ile971val	
QP5d-QP6	3247	A,G	AG	GG	AG	AA	GG	AG	AA	AA	GG	AG	AG	AG	AA	GG	AA	GG	GG	GG	AG	AG	AG	AG	AG	GG	GG	AA	AA	AG	AG	AG	AA	AG	AA	AG	AA	AA	41%	17/41 total	silent	50% in Japanese
QP7e-QP8	4459	G,A	AG	GG	AG	GG	AG	AG	GG	GG	AA	AG	AG	AG	GG	AG	GG	AG	AA	AG	AG	AG	AG	AG	AG	AA	GG	AG	AA		GG	AG	AA	GG	AA	AG	AA	AA	61%	21/41 total	3'UT	70% in Asians
QP7e-QP8	4506	G,A	AG	GG	AG	GG	AG	AG	GG	GG	AA	AG	AG	AG	GG	AG	GG	AG	AA	AG	AG	AG	AG	AG	AG	AA	GG	AG	AA		GG	AG	AA	GG	AA	AG	AA	AA	61%	21/41 total	3'UT	70% in Asians
			w	b	b	b	a	w,g	b	w	ash	h	cu,c	h	j	c	e	j	c	c	e	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w,i	67%	= Locus Heterozygosity		

ID#	Allele	Sequence around polymorphism*
VARIA300.1.1	2520 C	AATTCTGAACCTGCTGGTTTA
VARIA300.1.2	2520 A	AATTCTGAACATGCTGGTTTA
VARIA300.7.1	2944 G	TCATCACAAAGTCAGAAATGA
VARIA300.7.2	2944 A	TCATCACAAAATCAGAAATGA
VARIA300.2.1	2963 C	GATTGAATACCATGACATAAG
VARIA300.2.2	2963 T	GATTGAATACTATGACATAAG
VARIA300.3.1	2969 A	ATACCATGACATAAGTGGCTG
VARIA300.3.2	2969 G	ATACCATGACGTAAGTGGCTG
VARIA300.4.1	3247 A	AATGGGTACAATCACACAGAG
VARIA300.4.2	3247 G	AATGGGTACAGTCACACAGAG
VARIA300.5.1	4459 G	GATACAGACCGTTTTATGATT
VARIA300.5.2	4459 A	GATACAGACCATTTTATGATT
VARIA300.6.1	4506 G	AAGTCACACAGGACAATTATT
VARIA300.6.2	4506 A	AAGTCACACAAGACAATTATT

* Bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession # X54326
Fett, R. and R. Knippers (1991) The Primary Structure of Glutaminyl-tRNA Synthetase. *J. Biol. Chem.* 266: 1448-1455.*

*Note: See Kaiser,E., Hu,B., Becher,S., Eberhard,D., Schray,B., Baack,M., Hameister,H., and R. Knippers (1994) *Genomics* 19: 280-290 for the correct name of the gene. (The Fett and Knippers paper is mistaken.)

Validation:
Multiple other tRNA synthetases have been proven essential for cell survival.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

3/4/98

Target Gene Summary Table
Initiation Factor eIF-5A
Chromosome 17p13-p12
VARIA351

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals																																	Het%	Comments	Location	Race Specific Heterozygosity
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36				
eIF3-9	623	A,G	AA	GG	GG	AG	GG	AA	GG	GG	GG	AG	AG	AG	AG	GG	GG	GG	GG	GG	GG	GG	GG	AG	AG	GG	AG	AG	GG	AG	AG	GG	AG	AG	GG	GG	GG	GG	37%	1 nucleotide away from splice acceptor	3'UT	44% Caucasian
eIF11F-4	1012	C,T	CC	CC	TC	CC	TC	CC	TC	TC			CC	TT	TC	CC	TC	CC	TC	TC	CC	CC	CC	TC	TC	TC	TC	CC	TT	TC	CC	CC	TC	TC	TC	CC	TC	CC	52%		3'UT	present in all groups
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c	a	j	j	w	i	pr	w	w	w	w	w	w	w	w	w	w	w,h	83 % = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA351.1.1	623 A	GGCTCCCAGGATGGCGGTGGT
VARIA351.1.2	623 G	GGCTCCCAGGGTGGCGGTGGT
VARIA351.2.1	1012 C	CCCTGTTGCCCATAGCCCTTT
VARIA351.2.2	1012 T	CCCTGTTGCCTATAGCCCTTT

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession # U17969 (This accession contains the genomic sequence, with the location of intron-exon boundaries indicated. See Variagenics annotated cDNA sequence for sequence numbering used in this table.)

Koettnitz, K., Wohl, T., Kappel,B., Lottspeich, F., Hauber, J. and D. Bevec (1995) Identification of a new member of the human eIF-5A family. *Gene* 159: 283-284.

Validation:
This is the only human protein which contains hypusine. Inhibition of hypusine formation is cytostatic.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

3/4/98

Target Gene Summary Table
Cytidine Triphosphate Synthetase
Chromosome 1p34.1
VARIA259

			Genotypes of 36 unrelated individuals																																						Race Specific
Primer Pair	Location	Base	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Het%	Comments	Location	heterozygosity
F3-R3	576	A,G	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	6%	Low frequency		1/4 Chinese
F11-R11	2093	C,T	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	3%	Low frequency	3' UTR	1/1 Cambodian
F11-R11	2135	G,A	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GG	GG	GG	GA	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	8%	Low frequency	3' UTR	2/4 Chinese
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c	a	j	i	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w	w,it	11%=Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA259.1.1	576	GTCAGTTCCAATTCAAGGTCA
VARIA259.1.2	576	GTCAGTTCCAGTTCAAGGTCA
VARIA259.2.1	2093	CAGAACATCGCGATGGGAACC
VARIA259.2.2	2093	CAGAACATCGTGATGGGAACC
VARIA259.3.1	2135	TGTCCCCATCGGTCACCTTGT
VARIA259.3.2	2135	TGTCCCCATCAGTCACCTTGT
		*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:

GenBank Accession # X52142

Yamauchi, M., Yamauchi, N. and M. Meuth (1990). Molecular cloning of the human CTP sythetase gene by functional complementation with purified human metaphase chromosomes. *EMBO J.* 9: 2095-2099.

Validation:
Cells are poisoned by cyclopentenylcytosine, a specific inhibitor of CTPS

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:

Target Gene Summary Table
Cysteinyl-tRNA Synthetase
Chromosome 11p15.5
VARIA301

Primer Pair	Location	Base	Genotypes of 36 unrelated individuals 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Het%	Comments	Protein change	Race Specific Heterozygosity
C3c-C4	nt 1739	C,T	CT	CT	CT								CT	CT		CT	CT				CT	CT					CT			CT	CT	CT		CT		CT	CT		44%		pro822leu	53% White 50% Black 50% Chinese
			w	b	b	b	a	w,a	b	w	ash	h	w,g	h	j	c	c	j	c	c	a	j	j	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w	w,i	44% = Locus Heterozygosity			

ID number	Allele	Sequence around polymorphism*
VARIA301.1.1	1739 C	ACATCCTGCCCGAGCTTGGGG
VARIA301.1.2	1739 T	ACATCCTGCCTGAGCTTGGGG

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms:

#	%	area
1	6%	5'UT

Sequence from:
GenBank accession # L06845, *and*
Cruzen, M.E. and S.M. Arfin (1994) Nucleotide and *Deduced Amino Acid Sequence of Human Cysteinyl* tRNA Sequence. *DNA Sequence* 4: 243-248

*The Cruzen and Arfin paper is the source for nt 1-2048. Genbank accession #L06845 contains a further 423 nt at the 3' end, but lacks the 19 consecutive A residues after 2029 reported in *Cruzen and Arfin*.

Validation:
Multiple other tRNA synthetases have been proven essential for cell survival.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
Swedes: 37% heterozygosity (98/267)

Target Gene Summary Table
Alanyl-tRNA Synthetase
Chromosome 16q22
VARIA304

Primer Pair	Location	Base	Genotypes of 36 unrelated Individuals: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ala1a-Ala2	bp 1013	C,T	CT	CT	TT	CT	CT	CC	CT	CC	TT	CT	TT	TT	CT	CT	CT	CC	CC	CC
			w	b	b	b	a	w,g	b	w	ash	h	cu,g	h	j	c	c	j	c	c

Primer Pair	19	20	21	22	23	24	25	26	27	28	29	30	31	32	CT	34	35	36	Het%	Comments	Protein	Race Specific Herozygosity
Ala1a-Ala2	CT	CC	TT	CT	TT	CT	CT	CT	CT		CT	CT	CT	TT	CT	CC	CT	CC	57%		Silent	
	a	i	i	ar	i	pr	w	w	w	w	w	w	w	w	w	w	w	w,it	57% = Locus Heterozygosity			

ID#	Allele	Sequence around polymorphism*
VARIA304.1.1	1013 T	CTGGCTGACCATGCTCGGACCA
VARIA304.1.2	1013 C	CTGGCTGACCACGCTCGGACCA

*bold nucleotide is the polymorphic base

Other SSCP polymorphisms: # %

Sequence from:
GenBank accession # D32050, described in:
Shiba,K., Ripmaster, T., Suzuki,N., Nichols,R., Plotz,P., Noda, T. and P. Schimmel (1995) Human alanyl-tRNA synthetase: conservation in evolution of catalytic core and microhelix recognition. *Biochemistry* 34: 10340-10349.

Validation:
Multiple other tRNA synthetases have been proven essential for cell survival.

Ethnic & Racial Groups Surveyed:
a=Asian (other)
ar=Arab
ash=Ashkenazi
b=Black
c=Chinese
cu=Cuban
g=Greek
h=Hispanic
i=Indian
it=Italian
j=Japanese
pr=Puerto Rican
w=White
*empty box = genotype not determined

Other populations genotyped:
None

Fig. 2

SSCP Overview

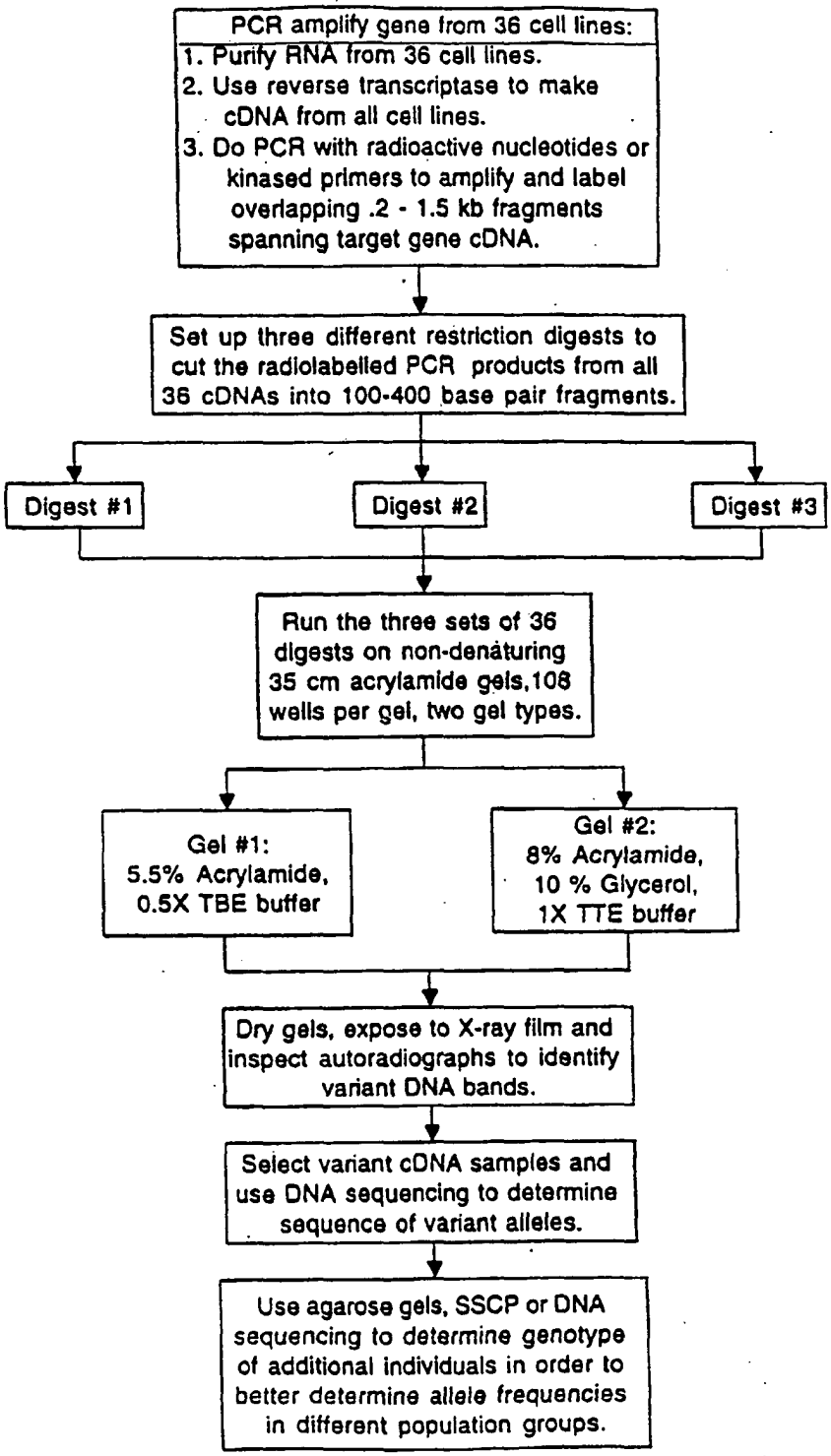

Fig.3
Chromosome 1 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
36	D1Z2	110	24	0.22	Breast	GCC 5:311
36	D1Z2	37	15	0.41	Breast	AJHG 45:73
36	D1Z2	18	9	0.5	Endocrine	CR 52:770
36	D1Z2	20	1	0.05	Endocrine	CR 52:770
36	D1Z2	7	7	1	Neuroblastoma	CR 55:5366
36	D1S243	43	10	0.23	Breast	CR 55:1752
36	D1S243	20	6	0.3	Endocrine	Unknown
36	D1S243	14	14	1	Neuroblastoma	CR 55:5366
36	D1S243	36	9	0.25	Neuroblastoma	CR 55:5681
36	D1S243	8	7	0.88	Neuroblastoma	GCC 10:275
36-35	D1S80	9	0	0	Brain	CR 54:1397
36-35	D1S80	14	1	0.07	Brain	CR 54:1397
36-35	D1S80	34	16	0.47	Brain	AJP 145:1175
36-35	D1S80	17	4	0.24	Breast	GCC 12:16
Unknown	D1S80	74	22	0.3	Breast	CR 53:1990
36-35	D1S80	63	20	0.32	Breast	CR 54:4274
36-35	D1S80	40	8	0.2	Endocrine	GCC 13:9
36-35	D1S80	13	10	0.77	Neuroblastoma	GCC 10:275
36-35	D1S80	38	9	0.24	Neuroblastoma	CR 55:5681
Unknown	D1S80	19	2	0.11	Testis	CR 54:6265
Unknown	D1S80	17	2	0.12	Testis	CR 54:6265
36.3-35	D1S76	34	16	0.47	Brain	AJP 145:1175
36.3-35	D1S76	41	4	0.1	Breast	CR 53:4356
36.3-35	D1S76	19	3	0.16	Breast	GCC 12:16
36.3-35	D1S76	38	13	0.34	Breast	CR 54:4274
36.3-35	D1S76	17	15	0.88	Neuroblastoma	GCC 10:275
Unknown	D1S77	21	10	0.48	Brain	AJP 145:1175
Unknown	D1S77	19	3	0.16	Breast	GCC 12:16
Unknown	D1S77	18	4	0.22	Esophageal	GCC 10:177
Unknown	D1S77	6	2	0.33	Stomach	BJC 73:424
Unknown	D1S253	17	3	0.18	Leukemia	CR 56:537
36	D1S47	32	3	0.09	Breast	CR 51:1020
36	D1S47	15	1	0.07	Colon	CR 52:205
36	D1S47	17	12	0.71	Colon	CR 50:7232
36	D1S47	24	7	0.29	Melanoma	PNAS 86:4814
36	D1S47	31	7	0.23	Neuroblastoma	GCC 10:30
36	D1S214	43	8	0.19	Breast	CR 55:1752
36	D1S214	11	10	0.91	Neuroblastoma	GCC 10:275

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36	D1S214	13	0	0	Stomach	BJC 75:422
Unknown	D1S160	17	9	0.53	Brain	AJP 11145:11
Unknown	D1S160	21	5	0.24	Liver	CR 54:4188
Unknown	D1S160	34	8	0.24	Neuroblastoma	CR 55:5681
Unknown	D1S160	41	22	0.54	Ovary	BJC 75:1105
Unknown	D1S244	36	9	0.25	Neuroblastoma	CR 55:5681
36	D1S450	37	8	0.22	Breast	CR 55:1752
Unknown	NPPA	1	0	0	Testis	GCC 13:249
Unknown	EGD	10	1	0.1	Testis	GCC 13:249
36	D1S228	40	5	0.12	Breast	CR 55:1752
36	D1S228	7	5	0.71	Neuroblastoma	GCC 10:275
36	D1S228	31	7	0.23	Neuroblastoma	CR 55:5681
36	D1S228	8	1	0.12	Stomach	BJC 75:422
Unknown	D1S170	19	5	0.26	Liver	CR 54:4188
Unknown	D1S170	36	7	0.19	Neuroblastoma	CR 55:5681
Unknown	D1S170	33	16	0.48	Ovary	BJC 75:1105
Unknown	D1S94	19	12	0.63	Colon	CR 50:7232
Unknown	D1S94	8	4	0.5	Neuroblastoma	0 7:1185
Unknown	D1S94	36	9	0.25	Neuroblastoma	GCC 10:30
35	D1S199	50	9	0.18	Breast	CR 55:1752
35	D1S199	30	4	0.13	Cervix	CR 56:197
35	D1S199	14	13	0.93	Neuroblastoma	CR 55:5366
35	D1S199	4	2	0.5	Neuroblastoma	GCC 10:275
35	D1S199	9	0	0	Stomach	BJC 73:424
36.1-p34	ALPL	17	2	0.12	Colon	CR 52:20
36.1-p34	ALPL	2	1	0.5	Endocrine	CR 52:770
36.1-p34	ALPL	17	4	0.24	Melanoma	PNAS 86:4014
36.11	D1S112	1	1	1	Neuroblastoma	CR 55:5366
Unknown	D1SB12	20	1	0.05	Neuroblastoma	0 7:1185
Unknown	FUCA1	15	5	0.33	Brain	AJP 1145:117
Unknown	FUCA1	13	6	0.46	Melanoma	PNAS 86:4014
Unknown	FUCA1	14	0	0	Testis	GCC 13:249
Unknown	D1S234	10	8	0.8	Neuroblastoma	GCC 10:275
36.2-36.1	FGR	12	2	0.17	Brain	CR 54:1397
36.2-36.1	FGR	7	0	0	Brain	CR 54:1397
36.2-36.1	FGR	4	2	0.5	Endocrine	CR 52:770
36.2-36.1	FGR	14	6	0.43	Ovary	BJC 75:1105

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Unknown	D1S63	39	4	0.1	Testis	CR 54:6265
Unknown	D1S247	2	1	0.5	Neuroblastoma	GCC 10:275
36.2-34	D1S95-96	74	20	0.27	Breast	CR 53:1990
Unknown	D1S95	17	11	0.65	Colon	CR 50:7232
36.2-36.12	D1S95	19	2	0.11	Neuroblastoma	0 7:1185
Unknown	D1S96	18	0	0	Neuroblastoma	0 7:1185
32	D1S7	105	43	0.41	Breast	CR 54:4274
32	D1S7	46	13	0.28	Breast	GCC 2:191
32	D1S7	28	26	0.93	Colon	CR 50:7232
32	D1S7	14	7	0.5	Endocrine	N 326:524
32	D1S7	13	1	0.08	Liver	BJC 64:1083
32	D1S7	50	15	0.3	Liver	BJCR 9:89
32	D1S7	6	6	1	Neuroblastoma	CR 55:5366
32	D1S7	14	5	0.36	Pancreas	BJC 65:809
32	D1S7	31	3	0.1	Stomach	HG 92:244
32	D1S7	45	14	0.31	Stomach	CR 51:2826
32	D1S7	31	3	0.1	Stomach	BJC 73:424
32	D1S7	30	1	0.03	Testis	GCC 13:244
Unknown	D1S233	19	5	0.26	Head&Neck	CR 54:1152
Unknown	D1S233	4	2	0.5	Neuroblastoma	GCC 10:275
Unknown	D1S241	4	3	0.75	Neuroblastoma	GCC 10:275
Unknown	D1S201	35	0	0	Head&Neck	CR 54:4756
Unknown	D1S201	19	1	0.05	Head&Neck	CR 54:4756
Unknown	D1S201	8	3	0.38	Neuroblastoma	GCC 10:275
Unknown	D1S201	12	3	0.25	Stomach	BJC 73:424
35-32	D1S57	15	1	0.07	Brain	CR 50:6284
32	D1S57	26	12	0.46	Brain	AJP 1145:117
35-32	D1S57	11	0	0	Brain	CR 49:6572
35-32	D1S57	18	1	0.06	Breast	GCC 2:191
35-32	D1S57	75	16	0.21	Breast	GCC 2:331
35-32	D1S57	43	4	0.09	Breast	CR 50:7184
35-32	D1S57	81	36	0.44	Breast	GCC 94:4274
35-32	D1S57	3	2	0.67	Breast	CR 53:3804
35-32	D1S57	14	6	0.14	Breast	CR 51:3030
35-32	D1S57	19	6	0.32	Breast	CR 51:6194
35-32	D1S57	23	5	0.22	Breast	GCC 12:16
32	D1S57	74	23	0.31	Breast	CR 53:1990
32	D1S57	52	1	0.02	Cervix	CR 54:4484
35-32	D1S57	6	0	0	Cervix	GCC 9:119
35-32	D1S57	180	40	0.22	Colon	BJC 64:475
35-32	D1S57	22	2	0.09	Colon	CCG 48:167

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35-32	D1S57	16	6	0.38	Colon	CRC 52:382
35-32	D1S57	12	0	0	Colon	N 331:273
32	D1S57	16	1	0.06	Endocrine	CR 52:770
32	D1S57	12	8	0.67	Endocrine	CR 52:770
35-32	D1S57	15	6	0.4	Endocrine	CRC 52:0
32	D1S57	27	8	0.3	Esophageal	CR 54:2996
32	D1S57	15	1	0.07	Kidney	CR 51:820
35-32	D1S57	22	1	0.05	Liver	CR 51:89
35-32	D1S57	28	5	0.18	Lung	CR 52:2478
32	D1S57	2	2	1	Neuroblastoma	CR 55:5366
32	D1S57	14	1	0.07	Ovary	CR 51:5113
35-32	D1S57	18	7	0.39	Ovary	O 7:1059
35-32	D1S57	4	0	0	Pancreas	CR 54:1610
35-32	D1S57	20	2	0.1	Sarcoma	CR 52:2419
32	D1S57	5	3	0.6	Stomach	BJC 72:424
35-32	D1S57	17	0	0	Testis	G 5:134
32	D1S57	42	2	0.05	Testis	CR 54:2255
32	D1S57	37	2	0.05	Testis	CR 54:6265
32	D1S57	8	2	0.25	Uterus	GCC 9:136
32	D1S57	11	1	0.09	Uterus	CR 51:5632
Unknown	D1S255	14	7	0.5	Neuroblastoma	GCC 10:1
Unknown	D1S255	5	4	0.8	Stomach	BJC 73:424
Unknown	D1S186	25	7	0.28	Liver	CR 54:4180
32	MYCL1	74	26	0.35	Breast	CR 53:1990
32	MYCL1	81	36	0.44	Breast	GCC 12:128
32	MYCL1	152	55	0.36	Breast	HG 85:101
32	MYCL1	59	23	0.39	Breast	CR 54:6776
32	MYCL1	17	2	0.12	Breast	AJHG 45:73
32	MYCL1	16	10	0.62	Colon	CR 50:242
32	MYCL1	20	2	0.1	Colon	CR 52:285
32	MYCL1	20	5	0.25	Colon	CRC 52:382
32	MYCL1	9	1	0.11	Endocrine	CR 52:770
32	MYCL1	20	2	0.1	Endocrine	GCC 9:349
32	MYCL1	12	8	0.67	Endocrine	CR 52:770
32	MYCL1	11	0	0	Esophageal	CR 54:2996
32	MYCL1	18	2	0.11	Liver	JJCR 81:108
32	MYCL1	27	0	0	Lung	CR 54:5643
32	MYCL1	5	0	0	Lung	CR 54:5643
32	MYCL1	11	1	0.09	Lung	CR 54:5643
32	MYCL1	57	12	0.21	Lung	O 10:937
32	MYCL1	20	2	0.1	Lung	PNAS 85:9250
32	MYCL1	2	1	0.5	Lung	CR 54:5643
Unknown	MYCL1	9	2	0.22	Neuroblastoma	CR 49:4095
32	MYCL1	41	9	0.22	Ovary	BJC 75:1105

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32	MYCL1	17	4	0.24	Ovary	GO 55:245
32	MYCL1	9	0	0	Sarcoma	CR 52:2419
32	MYCL1	1	0	0	Testis	CCG 52:72
32	MYCL1	20	1	0.05	Uterus	CR 54:4294
34.2-32.2	D1S190	23	3	0.13	Cervix	CR 56:197
Unknown	D1S193	7	2	0.29	Neuroblastoma	GCC 10:275
Unknown	D1S211	5	3	0.6	Neuroblastoma	GCC 10:275
Unknown	D1S197	16	5	0.31	Stomach	BJC 73:424
32	D1S62	15	0	0	Colon	CCG 48:167
Unknown	D1S162	0	5	0	Breast	Unknown
Unknown	D1S200	12	7	0.58	Neuroblastoma	GCC 10:275
Unknown	D1S15	74	22	0.3	Breast	CR 53:1990
Unknown	D1S15	24	6	0.25	Testis	CR 54:6266
pter-22	D1S21	74	20	0.27	Breast	CR 53:1990
31-pter	D1S21	12	1	0.08	Endocrine	CR 52:770
31-pter	D1S17	19	8	0.42	Brain	AJP 1145:117
31-pter	D1S17	5	0	0	Breast	CR 51:1020
pter-22	D1S17	4	3	0.75	Endocrine	CR 52:770
31-pter	D1S17	13	2	0.15	Endocrine	GCC 13:9
pter-22	D1S18	74	20	0.27	Breast	CR 53:1990

Chromosome 1 - p Arm

Unknown	D1S203	14	6	0.43	Neuroblastoma	GCC 10:275
Unknown	D1S216	11	0	0	Stomach	BJC 73:424
Unknown	D1S209	15	7	0.47	Neuroblastoma	GCC 10:275
Unknown	D1S159	16	3	0.19	Liver	CR 54:4188
Unknown	D1S219	8	0	0	Stomach	BJC 73:424
31	D1S468	44	11	0.25	Breast	CR 55:1752
21	D1S216	14	13	0.93	Neuroblastoma	CR 55:5366
21	D1S216	8	4	0.5	Neuroblastoma	GCC 10:275
pter-31	D1S2	12	7	0.58	Brain	AJP 145:1175
pter-31	D1S2	1	0	0	Breast	[illegible]
pter-31	D1S2	74	19	0.26	Breast	CR 53:1990
pter-31	D1S2	16	3	0.19	Melanoma	PNAS 86:4614
31	D1S500	33	8	0.24	Breast	CR 55:1752
31	D1S450	39	11	0.28	Breast	CR 55:1752
Unknown	D1S207	15	8	0.53	Neuroblastoma	GCC 10:275
Unknown	D1S207	14	2	0.14	Stomach	BJC 73:424
pter-22	D1S16	74	22	0.3	Breast	CR 53:1990
pter-22	D1S16	21	0	0	Cervix	CR 54:4481
pter-22	D1S16	6	2	0.33	Endocrine	CR 52:770
pter-22	D1S16	24	4	0.17	Melanoma	PNAS 86:4614
pter-22	D1S16	13	5	0.38	Testis	CR 54:6266
31	D1S225	36	7	0.19	Breast	CR 55:1752
Unknown	D1S167	9	1	0.11	Liver	CR 54:4188
Unknown	AF3	10	0	0	Breast	AJHG 45:73
Unknown	AF3	26	6	0.23	Testis	CR 54:6265
Unknown	D1S236	11	5	0.45	Neuroblastoma	GCC 10:275
22-13	D1S10	74	19	0.26	Breast	CR 53:1990
Unknown	AMY1A	17	0	0	Testis	CR 54:6265
21	AMY2B	16	5	0.31	Liver	CR 54:4188
21	AMY2B	16	3	0.19	Ovary	BJC 69:429
21	AMY2B	12	0	0	Uterus	CR 54:4294
22-13	D1S14	74	24	0.32	Breast	CR 53:1990
22-13	D1S14	18	3	0.17	Endocrine	GCC 13:9
22-13	D1S14	23	4	0.17	Testis	CR 54:6265
21-13	D1S73	13	6	0.46	Brain	AJP 145:1175
21-13	D1S73	74	23	0.31	Breast	CR 53:1990
21-13	D1S73	22	6	0.27	Breast	GCC 12:16
21-13	D1S73	23	6	0.26	Testis	CR 54:6265
22-13	D1S9	8	6	0.75	Brain	AJP 145:1175
22-13	D1S9	74	23	0.31	Breast	CR 53:1990
22-13	D1S9	25	0	0	Testis	CR 54:6265
22	HSPIA	18	1	0.06	Colon	CR 52:285

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13	D1S418	39	8	0.21	Breast	CR 55:1752
13	NRAS	74	21	0.28	Breast	CR 55:1990
13	NRAS	10	5	0.5	Endocrine	CR 52:770
13	NRAS	6	1	0.17	Endocrine	CR 52:770
13	NGFB	32	13	0.41	Brain	AJP 145:1175
13	NGFB	6	0	0	Breast	GCC 4:91
13	NGFB	13	2	0.15	Breast	AJHG 45:73
13	NGFB	13	9	0.69	Breast	CR 54:990
13	NGFB	18	3	0.17	Colon	IJC 53:382
13	NGFB	5	1	0.2	Testis	CCG 52:72
13	NGFB	16	0	0	Testis	CR 54:6266
13	NGFB	1	0	0	Testis	CCG 52:72
13	NGFB	3	0	0	Testis	CCG 52:72
13	NGFB	8	0	0	Uterus	CR 56:6264
22-13	D1S11	74	19	0.26	Breast	CR 53:1990
22-13	D1S36	12	2	0.17	Breast	PNAS 90:5760
22-13	D1S13	74	16	0.22	Breast	CR 53:1990
22-13	D1S13	7	6	0.86	Endocrine	CR 52:770
22-13	D1S64	18	10	0.56	Brain	AJP 149:1475
31-pter	Unknown	36	1	0.03	Breast	JNCI 84:506
32	D1S300-101	74	20	0.27	Breast	CR 54:1990
Unknown	D1S33	9	4	0.44	Breast	CR 51:1020
3-5	Unknown	37	6	0.16	Colon	BJC 69:450
Unknown	Unknown	14	0	0	Colon	CCG 48:167
Unknown	D1S196	23	4	0.17	Endocrine	GCC 3:9
Unknown	D1S19	4	2	0.5	Endocrine	CR 52:770
Unknown	PND	3	2	0.67	Endocrine	CR 52:770
Unknown	D1S252	19	3	0.16	Head&Neck	CR 54:1152
Unknown	D1S57-NGFB	21	4	0.19	Head&Neck	CR 52:1454
Unknown	D1S243-D1S228	22	1	0.05	Kidney	PNAS 92:2854
Unknown	D1S243-D1S228	6	0	0	Kidney	PNAS 92:2854
Unknown	D1S:243-228	33	3	0.09	Kidney	CR 55:6189
33-35	Unknown	14	2	0.14	Liver	BJC 67:900
Unknown	D1S187	19	4	0.21	Liver	CR 54:4188
Unknown	ISO1	27	6	0.22	Liver	CR 54:4188
Unknown	ISO2	13	4	0.31	Liver	CR 54:4188
Unknown	D1S15	21	6	0.29	Melanoma	CR 56:4644
Unknown	D1S:214-201-255	20	1	0.05	Melanoma	CR 56:589
Unknown	PND	13	5	0.38	Melanoma	PNAS 95:4641
Unknown	D1S220	20	10	0.5	Neuroblastoma	GCC 10:275
Unknown	D1S252	11	7	0.64	Neuroblastoma	GCC 10:275
Unknown	D1S252	8	2	0.25	Neuroblastoma	GCC 10:275

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Unknown	D1S97	10	0	0	Neuroblastom a	G/L:4185
Unknown	GGAT2A07	28	3	0.11	Neuroblastom a	CR 55:5681
Unknown	D1S80	16	1	0.06	Ovary	BJC 54:54A
Unknown	D1S:162-175	14	1	0.07	Ovary	BJC 72:1330
Unknown	PB-PMY	25	6	0.24	Ovary	CR 52:2956
Unknown	MTHFR	28	16	0.57	Ovary	BJC 75:1105
3.3-.56	PND-D1S2-NGE5	11	0	0	Prostate	G/L:530
3.3-.5	Unknown	9	3	0.33	Stomach	BJC 59:750
STM		7135	1686	0.26		

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Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D1S305	30	7	0.23	Cervix	CR 56:197
CENTR	D1S305	14	1	0.07	Neuroblastoma	CR 55:5366
Unknown	D1S67	30	1	0.03	Brain	AJP 145:1175
21	D1S67	74	7	0.09	Breast	CR 53:1990
Unknown	D1S67	15	2	0.13	Breast	CR 56:7186
Unknown	D1S67	7	2	0.29	Cervix	GCC 9:119
Unknown	D1S67	26	3	0.12	Esophageal	GCC 10:177
Unknown	D1S67	14	1	0.07	Kidney	CR 51:820
Unknown	D1S67	6	1	0.17	Lung	CR 52:2690
Unknown	D1S67	3	3	1	Lung	CR 52:2478
Unknown	D1S67	1	1	1	Lung	CR 52:2478
Unknown	D1S67	17	5	0.29	Lung	CR 52:2478
Unknown	D1S67	14	4	0.29	Ovary	CR 52:5418
21	D1S67	23	2	0.09	Ovary	IJC 54:546
Unknown	D1S67	26	3	0.12	Testis	CR 56:5265
Unknown	D1S67	22	4	0.18	Uterus	GCC 9:119
21-23	MUC1	74	9	0.12	Breast	CR 53:1990
21-23	MUC1	7	0	0	Breast	CR 53:3804
21-23	MUC1	44	13	0.3	Breast	GCC 12:16
21-23	MUC1	43	7	0.16	Breast	CR 51:1020
21-23	MUC1	21	7	0.33	Head&Neck	CR 56:1496
21-23	MUC1	16	4	0.25	Stomach	CR 51:2926
21-23	MUC1	25	2	0.08	Testis	GCC 13:249
21	PEM-pMUC10	89	14	0.16	Breast	GCC 5:311
21	SPTA1	74	9	0.12	Breast	CR 53:1990
21	SPTA1	6	2	0.33	Breast	GCC 12:16
21	SPTA1	6	2	0.33	Breast	EN 86:7203
21	SPTA1	22	2	0.09	Colon	CR 52:285
21	SPTA1	29	3	0.1	Colon	CR 52:285
Unknown	D1S176	17	1	0.06	Liver	CR 54:4188
22-25	ATP1B1	74	9	0.12	Breast	CR 53:1990
21-23	APOA2	6	0	0	Breast	GCC 2:191
21-23	APOA2	18	4	0.22	Ovary	BJC 69:429
21-23	APOA2	5	0	0	Testis	GCC 13:249
21-23	APOA2	26	2	0.08	Uterus	CR 56:4296
21-31	D1S61	74	10	0.14	Breast	CR 53:1990
21-31	D1S61	52	12	0.23	Breast	CR 54:6020
21-31	D1S61	39	8	0.21	Breast	GCC 12:16
21-31	D1S61	21	2	0.1	Endocrine	GCC 13:8
Unknown	D1S75	14	0	0	Brain	AJP 145:1175
Unknown	D1S75	18	1	0.06	Testis	CR 56:6285
Unknown	D1S66	14	4	0.29	Esophageal	CR 54:2996
Unknown	D1S66	11	0	0	Sarcoma	CR 52:2419
23-25	AT3	19	0	0	Brain	CR 54:1397
23-25	AT3	14	0	0	Brain	CR 54:1397

Chromosome 1 - q Arm

23-25	AT3	14	1	0.07	Breast	AJHG 45:73
23-25	AT3	21	0	0	Breast	GCC 2:191
23-25	AT3	14	0	0	Colon	CR 52:285
23-25	AT3	14	0	0	Liver	GCC [illegible]
23-25	AT3	22	1	0.05	Ovary	IJC 54:546
23-25	AT3	5	0	0	Ovary	CR 50:2224
23-25	AT3	27	0	0	Testis	CR 54:6265
23-25	AT3	8	2	0.25	Testis	GCC 13:249
Unknown	D1S238	22	4	0.18	Cervix	CR 56:197
31-32.1	F13B	9	0	0	Brain	CR 54:1397
31-32.1	F13B	15	0	0	Brain	CR 54:1397
31-32.1	F13B	12	1	0.08	Endocrine	GCC 13:9
31-32.1	F13B	13	0	0	Uterus	CR 54:4294
Unknown	D1S65	18	0	0	Brain	AJP 145:1175
Unknown	D1S65	18	5	0.28	Breast	GCC 12:16
Unknown	D1S65	6	0	0	Esophageal	CR [illegible]
Unknown	D1S65	16	2	0.12	Head&Neck	CR 52:1494
Unknown	D1S65	15	3	0.2	Testis	CR 54:6265
32 or 42	REN	11	0	0	Brain	AJP 145:1175
32 or 42	REN	12	3	0.25	Breast	CR 51:1020
32	REN	21	7	0.33	Breast	GCC 12:16
32 or 42	REN	6	1	0.17	Breast	CR 53:1990
32 or 42	REN	12	2	0.17	Cervix	CR 49:3598
32	REN	16	1	0.06	Colon	CR 52:285
32 or 42	REN	19	7	0.37	Colon	IJC 53:382
32 or 42	REN	8	0	0	Liver	PNAS 86:8852
32 or 42	REN	14	0	0	Liver	JJCR 81:108
32 or 42	REN	4	0	0	Neuroblastoma	CR 49:1095
32 or 42	REN	21	1	0.05	Ovary	IJC 54:546
32 or 42	REN	8	0	0	Prostate	[illegible] 530
32 or 42	REN	15	4	0.27	Stomach	CR 52:3099
32 or 42	REN	11	3	0.27	Testis	CR 54:6265
32 or 42	REN	6	0	0	Uterus	CR 51:5632
32	D1S249	12	0	0	Neuroblastoma	CR [illegible]:5366
Unknown	LAMB2	13	1	0.08	Testis	CR 54:6265
Unknown	D1S58	24	11	0.46	Breast	GCC 12:16
Unknown	D1S58	27	7	0.26	Cervix	CR 54:4481
Unknown	D1S58	15	0	0	Colon	GCC 8:167
Unknown	D1S58	21	4	0.19	Testis	CR 54:6265
Unknown	D1S58	23	5	0.22	Testis	[illegible]
Unknown	D1S81	32	0	0	Brain	AJP 145:1175
Unknown	D1S81	39	12	0.31	Breast	GCC 12:16
Unknown	D1S81	41	5	0.12	Breast	CR 53:4356
Unknown	D1S81	20	1	0.05	Liver	CR 51:89
Unknown	D1S213	30	6	0.2	Cervix	CR 56:197

Chromosome 1 - q Arm

Unknown	D1S251	31	1	0.03	Kidney	CR 54:6189
Unknown	D1S74	11	4	0.36	Breast	GCC 12:16
Unknown	D1S8	52	10	0.19	Breast	GCC 12:16
Unknown	D1S74	39	7	0.18	Cervix	CR 54:4481
Unknown	D1S8	9	0	0	Endometrium	CR 54:224
32-44	D1S103	18	2	0.11	Ovary	BJC 69:429
Unknown	D1S74	4	0	0	Kidney	CR 54:6189
Unknown	D1S74	50	3	0.06	Testis	CR 54:3983
Unknown	D1S74	54	3	0.06	Testis	CR 54:3983
Unknown	D1S8	31	2	0.06	Testis	GCC 13:249
Unknown	D1S8	31	2	0.06	Testis	GCC 13:249
21-23	Unknown	70	18	0.26	Breast	JNCI 84:506
21-23	Unknown	75	16	0.21	Breast	CR 54:6265
Unknown	DF3	43	6	0.14	Breast	IJC 61:1
4.2-.3	Unknown	34	4	0.12	Colon	BJC 59:750
2.1-.4	Unknown	27	3	0.11	Colon	BJC 59:750
Unknown	D1S102	12	1	0.08	Endocrine	GCC 12:9
Unknown	D1S215	11	2	0.18	Endocrine	CR 56:599
Unknown	D1S259	27	5	0.19	Head&Neck	CR 54:4756
Unknown	D1S304-212	43	6	0.14	Head&Neck	CR 54:4756
Unknown	D1S304-212	17	2	0.12	Head&Neck	CR 54:4756
Unknown	Unknown	8	3	0.38	Liver	BJC 64:1083
32-44	Unknown	13	3	0.23	Liver	BJC 64:1083
Unknown	Unknown	4	1	0.25	Liver	BJC 64:1083
Unknown	D1S:237-212	27	2	0.07	Melanoma	CR 54:589
Unknown	APOA2-D1S:158-103	14	0	0	Ovary	BJC 72:1330
Unknown	REN-D1S81	23	9	0.39	Ovary	CR 54:2391
Unknown	Unknown	13	2	0.15	Pancreas	BJC 65:809
32-44	Unknown	7	0	0	Pancreas	CR 54:2761
4.2-.3	Unknown	6	1	0.17	Stomach	BJC 59:750
2.1-.4	Unknown	10	5	0.5	Stomach	BJC 59:750
Unknown	AGT	52	3	0.06	Testis	CR 54:3983
Unknown	AGT	4	0	0	Testis	CR 54:3983
Unknown	CR2	21	3	0.14	Testis	CR 54:6265
Unknown	D1S180	5	0	0	Testis	CR 54:3983
Unknown	D1S180	50	7	0.14	Testis	CR 54:3983
Unknown	D1S235	0	0	0	Testis	CR 54:3983
Unknown	D1S235	39	4	0.1	Testis	CR 54:3983
SUM		2869	317	0.15		

Chromosome 2 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D2S44	7	1	0.14	Uterus	GCC 9:119
Unknown	Unknown	11	1	0.09	Brain	CR 50:5784
Unknown	D2S44	7	1	0.14	Breast	CR 53:3806
Unknown	D2S44	74	6	0.08	Breast	CR 53:4356
Unknown	D2S47	23	0	0	Breast	CR 50:7144
23-15	D2S6	27	3	0.11	Breast	GCC 2:191
23-15	D2S6	22	2	0.09	Breast	JNCI 84:1506
23-15	D2S6	42	5	0.12	Breast	CR 53:4356
23-PTPR	TPO	50	21	0.42	Breast	BCRT 32:5
Unknown	D2S139	27	4	0.15	Cervix	CR 56:197
Unknown	D2S177	18	2	0.11	Cervix	CR 56:197
Unknown	D2S44	7	0	0	Cervix	GCC 9:119
Unknown	D2S44	48	6	0.12	Cervix	CR 54:4481
Unknown	D2S48	26	3	0.12	Cervix	CR 54:4481
Unknown	APOB	7	0	0	Colon	GCG 48:167
Unknown	D2S44	236	37	0.16	Colon	BJC 64:475
Unknown	D2S45	14	0	0	Colon	GCG 48:167
Unknown	D2S155	11	2	0.18	Endocrine	CR 56:599
Unknown	D2S44	60	10	0.17	Esophageal	GCC 10:177
Unknown	D2S44	20	4	0.2	Esophageal	CR 54:2996
Unknown	D2S47	41	10	0.24	Esophageal	GCC 10:177
Unknown	D2S47	30	2	0.07	Esophageal	CR 54:2996
Unknown	D2S162	21	4	0.19	Head&Neck	CR 54:1152
Unknown	D2S166-149	15	0	0	Head&Neck	CR 54:4756
Unknown	D2S166-149	20	1	0.05	Head&Neck	CR 54:4756
Unknown	D2S207-D2S131	21	0	0	Kidney	PNAS 92:2854
Unknown	D2S207-D2S131	6	0	0	Kidney	PNAS 92:2854
Unknown	D2S47	11	2	0.18	Kidney	CR 51:820
Unknown	D2S207-131	12	0	0	Kidney	CR 55:6189
Unknown	D2S48	9	0	0	Liver	CR 51:89
13	TGFA	5	0	0	Liver	PNAS 86:8852
Unknown	Unknown	27	6	0.22	Lung	CR 54:2322
Unknown	D2S44	7	2	0.29	Lung	CR 54:5643
Unknown	D2S44	4	2	0.5	Lung	CR 54:5643
Unknown	D2S44	22	11	0.5	Lung	CR 54:5643
Unknown	D2S47	19	1	0.05	Lung	CR 522478
12	CD8A	20	3	0.15	Ovary	BJC 69:429
Unknown	D2S44	23	9	0.39	Ovary	CR 53:2393
Unknown	D2S47	11	0	0	Ovary	CR 51:511P
23-15	D2S6	31	7	0.23	Ovary	IJC 54:546
23-PTER	TPO	14	2	0.14	Ovary	BJC 69:429
Unknown	D2S1	14	1	0.07	Prostate	G 11:530
Unknown	D2S3-D2S6	6	0	0	Prostate	G 11:530
Unknown	D2S47	10	2	0.2	Sarcoma	CR 52:2419
Unknown	D2S123	13	1	0.08	Stomach	CR 55:1933
Unknown	D2S44	45	12	0.27	Testis	O 9:2245

Chromosome 2 - p Arm

Unknown	D2S48	31	5	0.16	Testis	O 9:2245
24	MYCN	2	0	0	Testis	CCG 52:72
24	MYCN	2	0	0	Testis	CCG 52:72
24	MYCN	2	0	0	Testis	CCG 52:72
13	D2S101	21	0	0	Uterus	CR 54:4294
Unknown	D2S44	7	1	0.14	Uterus	GCC 9:119
SOM		1272	191	0.15		

Chromosome 2 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
13	IL1A	20	0	0	Uterus	CR 54:4294
Unknown	D2S44	17	0	0	Brain	CR 49:6572
Unknown	D2S44	17	0	0	Brain	CR 50:5784
Unknown	CRYG	8	1	0.12	Breast	GCC 2:191
Unknown	D2S44	51	7	0.14	Breast	GCC 4:113
Unknown	D2S44	31	3	0.1	Breast	GCC 2:191
Unknown	D2S44	49	5	0.1	Breast	CR 50:7104
Unknown	CRYG	9	1	0.11	Cervix	CR 49:3598
Unknown	D2S122	28	4	0.14	Cervix	CR 56:197
Unknown	D2S172	29	7	0.24	Cervix	CR 56:197
Unknown	CRYG	21	0	0	Colon	N 331:273
35-37	D2S3	16	0	0	Colon	CCG 48:167
Unknown	D2S44	32	1	0.03	Colon	CCG 48:167
Unknown	D2S54	8	0	0	Colon	CCG 48:167
Unknown	D2S125	20	2	0.1	Endocrine	CR 56:599
Unknown	D2S44	14	1	0.07	Esophageal	CR 51:2113
Unknown	D2S55	13	0	0	Esophageal	CR 54:2996
Unknown	D2S111	20	3	0.15	Head&Neck	CR 54:1152
Unknown	D2S163	10	0	0	Head&Neck	CR 54:4756
Unknown	D2S163	20	4	0.2	Head&Neck	CR 54:4756
Unknown	D2S125	28	1	0.04	Kidney	PNAS 92:2854
Unknown	D2S44	38	5	0.13	Kidney	CR 51:820
33-35	CRYP1	1	0	0	Liver	CR 51:89
Unknown	D2S44	18	0	0	Liver	CR 51:89
Unknown	D2S44	4	0	0	Liver	PNAS 86:8852
p16-15	D2S5	4	0	0	Liver	CCG 48:72
Unknown	D2S44	40	11	0.28	Lung	CR 522478
p16-15	D2S5	1	0	0	Neuroblastoma	CR 49:1095
Unknown	D2S3	23	9	0.39	Ovary	CR 53:2393
Unknown	D2S44	29	4	0.14	Ovary	CR 51:5118
p16-15	D2S5	5	1	0.2	Ovary	CR 50:2724
Unknown	D2S50	10	0	0	Ovary	CR 50:2724
Unknown	D2S55	19	2	0.11	Ovary	IJC 54:546
Unknown	D2S72	16	6	0.38	Ovary	BJC 69:429
Unknown	D2S44	4	0	0	Pancreas	CR 54:2761
Unknown	D2S44	26	7	0.27	Sarcoma	CR 52:2419
Unknown	D2S44	18	1	0.06	Stomach	HG 92:244
Unknown	D2S44	27	0	0	Testis	LI 73:606
13	IL1A	20	0	0	Uterus	CR 54:4294
SUM		744	86	0.12		

Chromosome 3 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
26	D3S17	12	10	0.83	Kidney	CR 51:1071
26	D3S17	7	7	1	Lung	GCC 1:240
Unknown	D3S1307	36	2	0.06	Esophageal	BJC 73:366
Unknown	D3S1317	31	10	0.32	Kidney	BJC 69:230
Unknown	D3S1317	12	3	0.25	Stomach	CR 55:1933
25	D3S18	19	9	0.47	Kidney	CR 51:1071
25	D3S18	1	1	1	Lung	GCC 1:240
14	D3S1038	21	6	0.29	Esophageal	CR 54:6484
14	D3S1038	37	5	0.14	Esophageal	BJC 73:366
14	D3S1038	5	0	0	Kidney	GCC 12:76
14	D3S1038	40	19	0.47	Kidney	BJC 69:230
14	D3S1038	6	5	0.83	Lung	JAMA 273:55
14	D3S1038	1	1	1	Lung	JAMA 273:55
14	D3S1038	25	3	0.12	Uterus	CR 54:4294
Unknown	D3S1263	22	7	0.32	Cervix	CR 56:197
Unknown	D3S651	6	4	0.67	Kidney	CR 51:4707
Unknown	D3S651	18	3	0.17	Lung	CR 52:873
Unknown	D3S651	8	8	1	Lung	CR 52:873
24-25	RAF1	4	1	0.25	Breast	CR 53:3804
24-25	RAF1	3	1	0.33	Cervix	CR 49:3598
25	RAF1	10	10	1	Head&Neck	CGC 54:91
25	RAF1	1	0	0	Kidney	CR 51:4707
25	RAF1	22	20	0.91	Kidney	CR 51:1071
25	RAF1	12	9	0.75	Kidney	CR 51:1544
25	RAF1	2	2	1	Kidney	CR 51:1071
25	RAF1	22	10	0.45	Kidney	G 11:537
24-25	RAF1	17	9	0.53	Kidney	CR 49:1390
24-25	RAF1	4	2	0.5	Lung	GCC 1:95
24-25	RAF1	15	14	0.93	Lung	GCC 1:95
25	RAF1	1	1	1	Lung	CR 49:5130
24-25	RAF1	1	0	0	Lung	GCC 1:95
25	RAF1	5	5	1	Lung	O 4:451
25	RAF1	12	2	0.17	Prostate	G 11:530
25	RAF1	1	1	1	Uterus	CR 51:5632
24.2-26	D3S1286	37	12	0.32	Esophageal	IJC 69:1
Unknown	D3S1293	33	5	0.15	Esophageal	BJC 73:366
Unknown	D3S1293	40	2	0.05	Head&Neck	CR 54:4756
Unknown	D3S1293	39	10	0.26	Head&Neck	CR 54:4756
Unknown	D3S1020	5	5	1	Lung	CR 52:873
Unknown	D3S1020	7	3	0.43	Lung	CR 52:873
Unknown	D3S1002	5	5	1	Lung	CR 52:873
Unknown	D3S1002	12	3	0.25	Lung	CR 52:873
25.1	D3S669	22	3	0.14	Breast	CR 51:5794
25.1	D3S669	10	7	0.7	Kidney	CR 51:4707
Unknown	D3S669	5	5	1	Lung	CR 52:873
Unknown	D3S669	12	2	0.17	Lung	CR 52:873

Chromosome 3 - p Arm

Unknown	THRB	54	15	0.28	Breast	GCC 12:128
21-PTER	THRB	30	4	0.13	Breast	AJP 140:215
22-24.1	THRB	71	32	0.45	Breast	CR 54:3021
Unknown	THRB	24	9	0.38	Cervix	IJC 58:787
22-24.1	THRB	7	3	0.43	Cervix	CR 49:3598
24	THRB	9	1	0.11	Colon	IJC 53:382
24	THRB	44	10	0.23	Esophageal	BJC 73:366
24	THRB	9	3	0.33	Head&Neck	C 72:881
22-24.1	THRB	23	6	0.26	Head&Neck	CR 54:1152
22-24.1	THRB	3	0	0	Head&Neck	CGC 54:91
22-24.1	THRB	5	5	1	Kidney	CR 51:949
24	THRB	34	18	0.53	Kidney	G 11:537
22-24.1	THRB	11	11	1	Lung	CRC 49:5130
21-PTER	THRB	1	0	0	Lung	GCC 1:95
24	THRB	7	3	0.43	Lung	GCC 1:95
22-24.1	THRB	2	2	1	Lung	GCC 1:95
22-24.1	THRB	3	1	0.33	Lung	GCC 1:95
22-24.1	THRB	5	3	0.6	Lung	GCC 1:95
24	THRB	6	5	0.83	Lung	O 4:451
22-24.1	THRB	10	2	0.2	Lung	GCC 11:15
22-24.1	THRB	22	17	0.77	Lung	GCC 1:95
Unknown	THRB	38	22	0.58	Melanoma	GCC 15:102
24	THRB	22	5	0.23	Ovary	IJC 52:575
22-24.1	THRB	7	4	0.57	Ovary	O 5:219
Unknown	THRB	22	6	0.27	Ovary	IJC 54:546
22-24.1	THRB	17	5	0.29	Ovary	BJC 69:429
Unknown	THRB	16	0	0	Pediatric	CR 50:3279
24	THRB	11	0	0	Prostate	GCC 11:119
Unknown	THRB	2	0	0	Uterus	CR 51:5632
24	THRB	4	1	0.25	Uterus	CR 51:5632
24	RARB	5	3	0.6	Kidney	G 11:537
24.2-25	D3S1266	52	15	0.29	Esophageal	IJC 69:1
23	D3S647	24	2	0.08	Breast	CR 51:5794
23	D3S647	21	8	0.38	Esophageal	CR 54:6484
23	D3S647	30	4	0.13	Esophageal	BJC 73:366
23	D3S647	22	8	0.36	Kidney	BJC 69:230
23	D3S647	11	5	0.45	Kidney	CR 51:4707
pter-21	D3S12	5	0	0	Stomach	HG 89:445
22-24.2	D3S1211	17	4	0.24	Esophageal	IJC 69:1
21.3	D3S1029	23	4	0.17	Esophageal	CR 54:6484
21.3	D3S1029	1	1	1	Lung	JAMA 273:55
21.3	D3S1029	6	5	0.83	Lung	JAMA 273:55
Unknown	D3S867	18	5	0.28	Lung	CR 52:873
Unknown	D3S867	7	7	1	Lung	CR 52:873
Unknown	D3S1298	24	8	0.33	Cervix	CR 56:197
13	D3S685	54	6	0.11	Breast	CR 51:5794

Chromosome 3 - p Arm

Unknown	D3S685	6	3	0.5	Cervix	GCC 9:119
21.3-22	D3S1007	17	9	0.53	Esophageal	CR 54:6484
21.3-22	D3S1007	33	6	0.18	Esophageal	BJC 73:366
Unknown	D3S685	47	15	0.32	Esophageal	GCC 10:177
21.3-22	D3S1007	3	0	0	Kidney	GCC 12:76
Unknown	D3S685	27	18	0.67	Kidney	CR 51:4707
21.3-22	D3S1007	50	37	0.74	Lung	IJC 64:307
Unknown	D3S685	31	14	0.45	Lung	CR 52:873
Unknown	D3S685	10	10	1	Lung	CR 52:873
13	D3S685	1	1	1	Lung	CR 52:2478
13	D3S685	7	7	1	Lung	CR 52:2478
13	D3S685	3	3	1	Lung	CR 52:2478
13	D3S685	26	9	0.35	Lung	CR 52:2478
13	D3S685	18	3	0.17	Ovary	CR 51:5118
Unknown	D3S685	18	3	0.17	Ovary	CR 51:5118
Unknown	D3S685	11	2	0.18	Uterus	GCC 9:119
22-24.2	D3S1260	63	25	0.4	Esophageal	IJC 69:1
22-24.2	D3S1260	3	0	0	Melanoma	GCC 15:102
21	D3S11	16	0	0	Endocrine	CR 56:599
21	D3S11	7	4	0.57	Kidney	CR 49:1390
21	D3S2-S3	1	1	1	Breast	GCC 2:191
21	D3S2-S3	20	1	0.05	Breast	GCC 2:191
21	D3S2-S3	1	0	0	Breast	PN 84:2372
21	D3S2-S3	2	0	0	Breast	PN 84:2372
21	D3S2-S3	3	0	0	Breast	PN 84:2372
21.3	D3S686	34	2	0.06	Breast	CR 51:5794
21	D3S2	22	4	0.18	Cervix	CR 54:4481
Unknown	D3S2	16	6	0.38	Cervix	IJC 58:787
21	D3S2	9	9	1	Cervix	CR 49:3598
21	D3S2	16	3	0.19	Colon	IJC 53:382
21	D3S2	9	0	0	Colon	N 331:273
Unknown	D3S2	12	0	0	Endocrine	GCC 13:9
21	D3S2	22	8	0.36	Esophageal	CR 54:2996
Unknown	D3S2	10	1	0.1	Esophageal	CR 51:2113
21.3	D3S686	38	11	0.29	Esophageal	BJC 73:366
21	D3S2	4	3	0.75	Head&Neck	CGC 54:91
21	D3S2	14	6	0.43	Kidney	CR 51:949
Unknown	D3S2	2	0	0	Kidney	CR 51:1544
Unknown	D3S2	23	18	0.78	Kidney	CR 51:1071
Unknown	D3S2	2	1	0.5	Kidney	CGC 32:281
Unknown	D3S2	11	2	0.18	Kidney	PNAS 85:157
21	D3S2	14	8	0.57	Kidney	G 11:537
Unknown	D3S2	20	9	0.45	Kidney	CR 51:1544
14-21	D3S2	8	7	0.88	Kidney	CR 49:1390
21	D3S2	8	7	0.88	Kidney	N 327:721
21.3	D3S686	10	6	0.6	Kidney	CR 51:4707

Chromosome 3 - p Arm

Unknown	D3S2	4	1	0.25	Leukemia	CGC 61:42
21	D3S2	15	12	0.8	Lung	PNAS 84:925
21	D3S2	1	0	0	Lung	PNAS 84:925
21	D3S2	5	1	0.2	Lung	GCC 11:15
21	D3S2	5	2	0.4	Lung	GCC 1:95
Unknown	D3S2	1	0	0	Lung	N 329:451
21	D3S2	1	0	0	Lung	PNAS 84:925
21	D3S2	7	7	1	Lung	PNAS 84:925
21	D3S2	8	6	0.75	Lung	PNAS 86:509
Unknown	D3S2	9	8	0.89	Lung	N 329:451
Unknown	D3S2	1	0	0	Lung	N 329:451
21	D3S2	6	6	1	Lung	GCC 1:240
21	D3S2	6	5	0.83	Lung	PNAS 84:925
Unknown	D3S2	20	8	0.4	Lung	JJCR 80:924
Unknown	D3S2	6	5	0.83	Lung	NEJ 317:110
Unknown	D3S2	4	3	0.75	Lung	NEJ 317:110
Unknown	D3S2	2	1	0.5	Lung	NEJ 317:110
Unknown	D3S2	12	0	0	Lung	PNAS 84:925
21	D3S2	9	4	0.44	Lung	PNAS 86:509
21	D3S2	12	8	0.67	Lung	JJCR 80:924
21	D3S2	3	1	0.33	Lung	GCC 1:95
21	D3S2	11	8	0.73	Lung	GCC 1:95
21	D3S2	8	8	1	Lung	CR 49:5130
14-21	D3S2	5	5	1	Lung	GCC 5:119
21.3	D3S686	6	6	1	Lung	CR 52:873
21.3	D3S686	11	7	0.64	Lung	CR 52:873
Unknown	D3S2	11	6	0.55	Melanoma	GCC 15:102
Unknown	D3S2	6	0	0	Neuroblastoma	CR 49:1095
21	D3S2	16	1	0.06	Ovary	IJC 54:546
21	D3S2	6	4	0.67	Sarcoma	CGC 53:45
21	D3S2	12	4	0.33	Sarcoma	CR 52:2419
Unknown	D3S2	10	0	0	Stomach	CR 48:2988
Unknown	D3S2	19	1	0.05	Testis	G 9:2245
21	D3S2	12	4	0.33	Testis	G 5:134
Unknown	D3S2	5	0	0	Uterus	CR 51:5632
14.2	D3S3	1	0	0	Breast	GCC 2:191
14.2	D3S3	9	9	1	HeadNeck	CGC 54:91
14.2	D3S3	4	3	0.75	Kidney	CR 51:1071
14.2	D3S3	1	1	1	Kidney	CR 49:1090
14.2	D3S3	9	0	0	Kidney	PNAS 85:157
14.2	D3S3	2	1	0.5	Kidney	N 327:721
14.2	D3S3	3	1	0.33	Kidney	G 11:537
14.2	D3S3	6	3	0.5	Lung	GCC 1:95
14.2	D3S3	1	1	1	Lung	GCC 1:95
14.2	D3S3	4	4	1	Lung	GCC 1:240
14.2	D3S3	1	0	0	Lung	N 329:451

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14.2	D3S3	9	6	0.67	Lung	N 329:451
14.2	D3S3	3	3	1	Lung	GCC 1:95
14.2	D3S3	1	0	0	Lung	N 329:451
14.2	D3S3	2	1	0.5	Lung	NEJ 317:110
14.2	D3S3	4	3	0.75	Lung	NEJ 317:110
14.2	D3S3	4	0	0	Lung	GCC 11:15
14.2	D3S3	1	1	1	Lung	GCC 1:95
21.2-14.2	D3S32	8	0	0	Brain	CR 49:6572
21.2-14.2	D3S32	18	2	0.11	Brain	CR 50:5784
21.2-14.2	D3S32	16	3	0.19	Breast	CR 50:7184
21.2-14.2	D3S32	44	9	0.2	Breast	CR 51:5794
21.2-14.2	D3S32	30	12	0.4	Cervix	CR 54:4481
14.2-21.2	D3S32	3	3	1	Cervix	GCC 9:119
21.2-14.2	D3S32	17	7	0.41	Cervix	IJC 58:787
14.2-21.2	D3S32	4	1	0.25	Cervix	IJGC 67:71
14.2-21.2	D3S32	19	8	0.42	Esophageal	CR 54:2996
21.2-14.2	D3S32	28	10	0.36	Esophageal	BJC 73:966
21.2-14.2	D3S32	7	0	0	Head&Neck	C 72:881
21.2-14.2	D3S32	15	8	0.53	Kidney	CR 51:836
14.2-21.2	D3S32	15	9	0.6	Kidney	CR 51:4707
14.2-21.2	D3S32	21	17	0.81	Kidney	CR 51:1071
21.2-14.2	D3S32	18	8	0.44	Kidney	CR 51:949
21.2-14.2	D3S32	20	2	0.1	Liver	CR 51:89
21.2-14.2	D3S32	11	6	0.55	Lung	GCC 3:358
21.2-14.2	D3S32	17	11	0.65	Lung	CR 52:873
21.2-14.2	D3S32	6	6	1	Lung	O 4:451
21.2-14.2	D3S32	5	1	0.2	Lung	GCC 11:15
21.2-14.2	D3S32	4	4	1	Lung	CR 52:873
21.2-14.2	D3S32	17	10	0.59	Melanoma	GCC 15:102
21.2-14.2	D3S32	13	2	0.15	Ovary	IJC 54:546
21.2-14.2	D3S32	17	3	0.18	Ovary	CR 51:5118
21.2-14.2	D3S32	17	3	0.18	Ovary	CR 51:5118
21.2-14.2	D3S32	3	1	0.33	Pancreas	CR 54:2761
21.2-14.2	D3S32	10	1	0.1	Prostate	PNAS 87:875
21.2-14.2	D3S32	10	1	0.1	Prostate	Conveys 11
21.2-14.2	D3S32	33	15	0.45	Testis	O 9:2245
21.2-14.2	D3S32	4	2	0.5	Uterus	GCC 9:119
21.2-21.1	D3S1289	15	5	0.33	Melanoma	GCC 15:102
21.32-21.33	D3S643	14	4	0.29	Breast	CR 51:5794
21.32-21.33	D3S643	19	0	0	Esophageal	CR 54:6484
21.32-21.33	D3S643	3	3	1	Kidney	CR 51:4707
21.32-21.33	D3S643	17	4	0.24	Leukemia	B 83:3449
21.32-21.33	D3S643	6	3	0.5	Lung	CR 52:873
21.32-21.33	D3S643	3	3	1	Lung	CR 52:873
21	D3F15S2	15	7	0.47	Breast	CR 53:554
21	D3F15S2	33	5	0.15	Breast	CR 53:4356

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21	D3F15S2	2	0	0	Cervix	CR 49:3590
21	D3F15S2	5	3	0.6	Cervix	IJC 58:787
21	D3F15S2	21	17	0.81	Esophageal	BJC 308:746
21	D3F15S2	12	9	0.75	Head&Neck	C 72:881
21	D3F15S2	4	2	0.5	Head&Neck	CGC 54:91
21	D3F15S2	3	3	1	Kidney	CGC 32:281
21	D3F15S2	3	0	0	Kidney	
21	D3F15S2	24	14	0.58	Kidney	G 11:537
21	D3F15S2	7	1	0.14	Kidney	
21	D3F15S2	13	10	0.77	Kidney	CR 49:1390
21	D3F15S2	21	16	0.76	Kidney	PNAS 85:157
21	D3F15S2	9	9	1	Kidney	N 327:721
21	D3F15S2	2	1	0.5	Kidney	CR 51:949
21	D3F15S2	16	12	0.75	Kidney	
21	D3F15S2	0	0	0	Lung	N 329:451
21	D3F15S2	9	9	1	Lung	N 329:451
21	D3F15S2	7	3	0.43	Lung	GCC 11:157
21	D3F15S2	1	0	0	Lung	N 329:451
21	D3F15S2	7	2	0.29	Lung	CL 51:133
21	D3F15S2	8	3	0.38	Lung	PNAS 86:509
21	D3F15S2	8	2	0.25	Lung	GCC 3:358
21	D3F15S2	6	3	0.5	Lung	PNAS 86:509
21	D3F15S2	2	0	0	Lung	PNAS 86:509
21	D3F15S2	2	0	0	Lung	CL 51:133
21	D3F15S2	5	4	0.8	Lung	O 4:451
21	D3F15S2	1	0	0	Lung	GCC 1:95
21	D3F15S2	5	3	0.6	Lung	NEJ 317:1110
21	D3F15S2	7	4	0.57	Lung	GCC 1:95
21	D3F15S2	1	0	0	Lung	GCC 1:95
21	D3F15S2	2	2	1	Lung	CR 49:5130
21	D3F15S2	16	11	0.69	Lung	GCC 1:95
21	D3F15S2	12	7	0.58	Melanoma	GCC 15:102
21	D3F15S2	8	1	0.12	Ovary	O 5:219
21	D3F15S2	22	4	0.18	Ovary	IJC 52:575
21	D3F15S2	22	4	0.18	Ovary	IJC 54:546
21	D3F15S2	12	2	0.17	Ovary	BJC 69:429
21	D3F15S2	3	0	0	Testis	CCG 52:72
21	D3F15S2	1	0	0	Testis	CCG 52:72
21	D3F15S2	2	0	0	Testis	CCG 52:72
21	D3F15S2	18	2	0.11	Testis	GCC 13:249
21	D3F15S2	2	0	0	Uterus	CR 51:5632
Unknown	D3S1076	29	2	0.07	Esophageal	BJC 73:366
Unknown	D3S1076	14	4	0.29	Esophageal	CR 54:6484
Unknown	D3S1076	22	13	0.59	Kidney	BJC 69:230
Unknown	D3S965	1	0	0	Lung	CR 52:873
Unknown	D3S965	1	1	1	Lung	CR 52:873

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21.2	D3S660	33	0	0.18	Breast	CR 51:5794
Unknown	D3S660	6	2	0.33	Kidney	CR 51:4707
Unknown	D3S660	12	5	0.42	Lung	CR 52:873
Unknown	D3S660	8	8	1	Lung	CR 52:873
Unknown	D3S717	6	3	0.5	Kidney	CR 51:4707
Unknown	D3S717	4	2	0.5	Lung	CR 52:873
Unknown	D3S717	4	4	1	Lung	CR 52:873
Unknown	D3S936	11	4	0.36	Kidney	CR 51:4708
Unknown	D3S936	12	5	0.42	Lung	CR 52:873
Unknown	D3S936	4	4	1	Lung	CR 52:873
14.2-21.1	D3S1313	54	11	0.2	Esophageal	IJC 69:1
14.2-21.1	D3S1300	53	19	0.36	Esophageal	IJC 69:1
14.2-14.3	D3S678	50	19	0.38	Breast	CR 51:5794
14.2-14.3	D3S678	10	7	0.7	Kidney	CR 51:4707
Unknown	D3S687	25	8	0.32	Breast	CR 51:5794
Unknown	D3S687	13	8	0.62	Kidney	CR 51:4707
Unknown	D3S687	4	4	1	Lung	CR 52:873
Unknown	D3S687	15	3	0.2	Lung	CR 52:873
Unknown	D3S1228	31	4	0.13	Esophageal	BJC 73:366
25	D3S1228	18	8	0.44	Esophageal	CR 54:6484
25	D3S1228	26	12	0.46	Kidney	BJC 69:230
25	D3S1228	6	4	0.67	Lung	JAMA 273:55
25	D3S1228	1	1	1	Lung	JAMA 273:55
14.1-14.2	D3S1285	47	18	0.38	Esophageal	IJC 69:1
14.1-14.2	D3S1285	10	7	0.7	Melanoma	GCC 15:102
Unknown	D3S714	24	1	0.04	Breast	CR 51:5794
Unknown	D3S714	9	3	0.33	Lung	CR 52:873
14-13	D3S1217	28	18	0.64	Esophageal	C 73:2472
14-13	D3S1217	28	18	0.64	Head&Neck	CR 73:2472
Unknown	D3S1079	25	4	0.16	Esophageal	BJC 73:366
Unknown	D3S1079	11	4	0.36	Esophageal	CR 54:6484
Unknown	D3S1261	20	8	0.4	Cervix	CR 56:197
Unknown	D3S13	2	0	0	Stomach	JG 89:445
12-14.2	D3S1296	57	17	0.3	Esophageal	IJC 69:1
Unknown	D3S659	54	23	0.43	Breast	CR 51:5794
Unknown	D3S659	7	6	0.86	Cervix	GCC 9:119
Unknown	D3S659	28	10	0.36	Esophageal	GCC 10:177
Unknown	D3S659	36	6	0.17	Esophageal	BJC 73:366
Unknown	D3S659	17	7	0.41	Esophageal	CR 54:6484
Unknown	D3S659	11	8	0.73	Kidney	CR 51:4707
Unknown	D3S659	40	18	0.45	Kidney	BJC 69:230
Unknown	D3S659	17	5	0.29	Lung	CR 52:873
Unknown	D3S659	10	9	0.9	Lung	CR 52:873
Unknown	D3S659	6	0	0	Ovary	CR 51:5118
Unknown	D3S659	6	0	0	Ovary	CR 51:5118
Unknown	D3S659	11	5	0.45	Uterus	GCC 9:119

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Unknown	D3S659	14	1	0.07	Uterus	CR 54:429
13	D3S693	6	0	0	Breast	CR 51:5794
13	D3S693	5	0	0	Lung	CR 52:873
14	D3S6	32	11	0.34	Breast	CR 54:499
14	D3S6	5	2	0.4	Kidney	CR 49:1390
14	D3S6	3	0	0	Kidney	PNAS 85:157
14	D3S6	3	1	0.33	Kidney	GCC 11:65
14	D3S6	8	7	0.88	Lung	GCC 1:95
14	D3S6	6	2	0.33	Lung	GCC 1:95
14	D3S6	4	2	0.5	Lung	GCC 11:15
2L-3	ITPR1.R8	66	55	0.83	Lung	IJC 64:691
Unknown	D3S30	37	15	0.41	Breast	CR 54:3021
13	D3S30	18	0	0	Breast	CR 48:2167
Unknown	D3S30	17	6	0.35	Cervix	IJC 58:787
Unknown	D3S30	19	7	0.37	Esophageal	CR 54:2946
13	D3S30	32	12	0.38	Esophageal	BJC 73:366
Unknown	D3S30	16	8	0.5	Kidney	CR 51:3900
13	D3S30	18	9	0.5	Kidney	CR 51:820
Unknown	D3S30	12	3	0.25	Lung	CR 52:873
13	D3S30	7	1	0.14	Lung	GCC 11:15
Unknown	D3S30	11	11	1	Lung	CR 52:873
13	D3S30	7	7	1	Lung	GCC 1:240
Unknown	D3S30	11	8	0.73	Melanoma	GCC 15:107
13	D3S30	14	1	0.07	Ovary	CR 51:5118
13	D3S30	14	1	0.07	Ovary	CR 51:5118
Unknown	D3S30	12	1	0.08	Ovary	BJC 69:429
13	D3S30	18	0	0	Testis	G 31:134
13-14	D3S1284	19	12	0.63	Head&Neck	CR 54:1152
13-14	D3S1284	3	0	0	Kidney	GCC 12:15
Unknown	D3S738	3	3	1	Lung	GCC 5:119
Unknown	D3S625	2	2	1	Lung	GCC 5:119
Unknown	D3S742	4	3	0.75	Lung	GCC 5:119
Unknown	D3S739	5	3	0.6	Lung	GCC 5:119
Unknown	D3S740	5	4	0.8	Lung	GCC 5:119
Unknown	D3S216	1	1	1	Lung	GCC 5:119
Unknown	D3S733	3	3	1	Lung	GCC 5:119
13	D3S4	16	7	0.44	Kidney	CR 51:949
13	D3S4	17	4	0.24	Kidney	CR 51:1071
13	D3S4	14	8	0.57	Kidney	CR 49:1390
13	D3S4	6	5	0.83	Lung	GCC 1:240
Unknown	D3S743	5	4	0.8	Lung	GCC 5:119
Unknown	D3S759	7	6	0.86	Lung	GCC 5:119
Unknown	D3S640	5	3	0.6	Lung	GCC 5:119
Unknown	D3S1090	2	2	1	Lung	GCC 5:119
Unknown	D3S1090	2	2	1	Lung	GCC 5:119
Unknown	D3S:1067-1228	29	9	0.31	Bladder	CR 55:5213

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Unknown	RAF1-DNF15S2	25	17	0.68	Bladder	CR 51:5405
24-26	Unknown	28	13	0.46	Breast	JNCI 84:506
Unknown	D3S2-B3H2	37	12	0.32	Breast	CR 54:3021
Unknown	DNF15S2	4	1	0.25	Breast	CR 53:3804
24	ERBA-B	67	26	0.39	Breast	CR 54:499
Unknown	RAF1-DNF15S2	15	7	0.47	Breast	GE 5:554
Unknown	D3S663	6	3	0.5	Cervix	GCC 19:119
21.1-14.2	D3S1067	20	7	0.35	Esophageal	CR 54:6484
Unknown	D3S1110	17	7	0.41	Esophageal	CR 54:6484
Unknown	D3S1111	11	1	0.09	Esophageal	CR 54:6484
Unknown	D3S192	34	8	0.24	Esophageal	BJC 73:366
Unknown	D3S656	19	8	0.42	Esophageal	CR 54:6484
Unknown	D3S663	22	2	0.09	Esophageal	CR 54:6484
Unknown	D3S966	38	9	0.24	Esophageal	BJC 73:366
Unknown	D3S966	19	5	0.26	Esophageal	CR 54:6484
21.1-14.2	D3S1067	41	20	0.49	Kidney	BJC 69:230
25-26	D3S1085	3	3	1	Kidney	CR 51:4707
Unknown	D3S1110	15	11	0.73	Kidney	BJC 69:230
Unknown	D3S1263-D3S1307-D3S1297	22	9	0.41	Kidney	PNAS 92:285
Unknown	D3S1263-D3S1307-D3S1297	6	0	0	Kidney	PNAS 92:285
Unknown	D3S22	9	7	0.78	Kidney	CR 51:1071
25	D3S649	11	7	0.64	Kidney	CR 51:4707
Unknown	D3S654	13	4	0.31	Kidney	CR 51:4707
Unknown	D3S656	7	4	0.57	Kidney	CR 51:4707
25	D3S689	1	0	0	Kidney	CR 51:4707
25-26	D3S858	11	5	0.45	Kidney	CR 51:4707
21.1-21.2	D3S898	8	7	0.88	Kidney	CR 51:4707
14.1-14.2	D3S907	6	2	0.33	Kidney	CR 51:4707
12	D3S960	2	2	1	Kidney	CR 51:4707
Unknown	D3S:1263-1307-1297	33	10	0.3	Kidney	CR 55:6189
Unknown	DNF15S2	28	25	0.89	Kidney	CR 51:1071
Unknown	DNF15S2	19	9	0.47	Kidney	CR 51:1544
Unknown	ERBA-B	18	17	0.94	Kidney	CR 51:1071
Unknown	ERBA-B	2	0	0	Kidney	CR 51:1071
Unknown	RAF1-DNF15S2	13	7	0.54	Kidney	CR 51:949
25-26	VHL	19	16	0.84	Kidney	CR 54:2852
Unknown	Unknown	27	25	0.93	Lung	CR 54:2322
21.3	D3S1339	12	11	0.92	Lung	IJC 64:371
21	D3S48	5	5	1	Lung	GCC 5:119
Unknown	D3S654	9	7	0.78	Lung	CR 52:873
Unknown	D3S654	22	8	0.36	Lung	CR 52:873
Unknown	DNF15S2	5	1	0.2	Lung	NEJ 317:110
Unknown	DNF15S2	2	1	0.5	Lung	NEJ 317:110
Unknown	DNF15S2	5	5	1	Lung	NEJ 317:110

Chromosome 3 - p Arm

Unknown	ITIH1-D3S1339	7	7	1	Lung	CR 55:5133
	100%					
Unknown	RAF1-DNF15S2	4	4	1	Lung	GCC 5:119
Unknown	RAF1-DNF15S2	6	3	0.5	Lung	PNAS 86:509
Unknown	RAF1-DNF15S2	5	3	0.6	Lung	PNAS 86:509
Unknown	RAF1-DNF15S2	17	8	0.47	Lung	GCC 3:358
25-24	D3S1252	5	1	0.2	Melanoma	GCC 15:102
all	7 loci	46	11	0.24	Ovary	CR 53:4456
21	D3S2-D3S86	23	0	0	Ovary	CR 53:2393
Unknown	D3S1270-D3	14	2	0.14	Ovary	BJC 72:1330
Unknown	Unknown	19	2	0.11	Testis	G 5:134
21.1-14.2	D3S1067	25	3	0.12	Uterus	CR 54:4294
Unknown	D3S663	10	2	0.2	Uterus	GCC 9:119
SUM		5933	2353	0.4		

Chromosome 3 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Refere
11.0-12.0	GPX1	19	17	0.89	Kidney	Cr 15
11.0-12.0	GPX1	6	6	1	Lung	Cr 15
11.0-12.0	GPX1	3	3	1	Lung	Cr 15
12	D3S1	7	0	0	Head&Neck	CGC 5
12	D3S1	2	0	0	Kidney	CGC 3
12	D3S1	4	0	0	Lung	NEJ 3
12	D3S1	3	0	0	Lung	O 4,1
12	D3S1	1	0	0	Lung	N 329
12	D3S1	9	2	0.22	Lung	N 329
12	D3S1	1	0	0	Lung	N 329
12	D3S1	19	2	0.11	Ovary	BJC 6
12	D3S1	8	1	0.12	Testis	GCC 1
Unknown	D3S1764	24	1	0.04	Esophageal	BJC 7
Unknown	D3S196	31	3	0.1	Esophageal	BJC 7
Unknown	D3S196	19	9	0.47	Head&Neck	CR 54
Unknown	D3S196	19	5	0.26	Ovary	BJC 6
Unknown	D3S196	22	2	0.09	Uterus	CR 54
Unknown	CP	7	1	0.14	Lung	N 329
Unknown	CP	3	0	0	Lung	N 329
Unknown	CP	1	0	0	Lung	N 329
Unknown	D3S1268	24	2	0.08	Head&Neck	CR 54
Unknown	D3S1268	34	0	0	Head&Neck	CR 54
Unknown	D3S1268	35	5	0.14	Melanoma	CR 56
Unknown	D3S1262	37	8	0.22	Cervix	CR 56
Unknown	D3S1262	18	1	0.06	Esophageal	CR 54
28	SST	6	0	0	Cervix	CR 49
28	SST	6	0	0	Liver	CCC
28	SST	9	2	0.22	Lung	N 329
28	SST	17	0	0	Lung	PNAS
28	SST	1	0	0	Lung	N 329
28	SST	7	0	0	Lung	CR 49
28	SST	1	0	0	Melanoma	N 329
28	SST	3	0	0	Neuroblastoma	CR 49
Unknown	D3S1314	26	1	0.04	Kidney	PNAS
Unknown	D3S42	4	1	0.25	Breast	CR 53
Unknown	D3S42	26	3	0.12	Breast	GCC 4
Unknown	D3S42	28	9	0.32	Cervix	CR 54
Unknown	D3S42	12	0	0	Stomach	HG 92
Unknown	D3S42	34	9	0.26	Testis	O 9,2
Unknown	D3S42	16	0	0	Testis	LI 73
Unknown	D3S44	38	6	0.17	Ovary	CR 53
Unknown	D3S46	19	5	0.26	Esophageal	CR 54
Unknown	D3S46	0	32	0	Esophageal	Unkno
Unknown	D3S46	44	13	0.3	Esophageal	GCC 1
Unknown	D3S46	16	3	0.19	Kidney	CR 51

Chromosome 3 - q Arm

Unknown	D3S46	7	0	0	Liver	CR 51
Unknown	D3S46	40	6	0.15	Lung	GCC 23
Unknown	D3S46	18	1	0.06	Ovary	CR 51
Unknown	D3S46	18	1	0.06	Ovary	BJC 7
Unknown	D3S46	3	0	0	Pancreas	CR 54
Unknown	D3S46	12	9	0.75	Sarcoma	GCC 5
Unknown	D3S46	12	9	0.75	Sarcoma	CR 52
Unknown	Unknown	15	0	0	Brain	GCC 6
21-qter	D3S5	1	0	0	Brain	CCG 5
Unknown	MOX2	1	0	0	Brain	GCC 5
Unknown	D3S47	21	0	0	Endocrine	GCC 1
Unknown	GLUT2	23	0	0	Endocrine	GCC 6
Unknown	D3S1271	14	1	0.07	Esophageal	CR 54
Unknown	D3S1238	20	7	0.35	Esophageal	GCC 4
Unknown	D3S1-MOX2-D3S5	24	2	0.08	Kidney	G 11:
Unknown	D3S5	14	0	0	Kidney	GCC 9
26.2-qTER	D3S45	20	3	0.15	Kidney	CR 51
all	4 markers	32	15	0.47	Lung	GCC 1
12-q13	MOX1	15	7	0.47	Lung	GCC 1
12-q13	MOX1	6	2	0.33	Lung	GCC 1
12-q13	MOX1	1	1	1	Lung	GCC 1
12-q13	MOX1	1	1	1	Lung	GCC 1
all	4 markers	46	8	0.17	Ovary	CR 53
21-PTER	AGCP	13	4	0.31	Ovary	BJC 6
Unknown	D3S1232-GLUT2	14	2	0.14	Ovary	BJC 7
Unknown	D3S3	13	0	0	Prostate	G 11:
SUM		1050	191	0.18		

Chromosome 4 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
16.1	RAF1P1	7	0	0	Uterus	CR 51:5632
Unknown	D4S1546	25	8	0.32	Bladder	CR 55:5213
Unknown	D4S124	16	0	0	Brain	CR 50:5784
16	D4S10	31	0	0	Breast	GE 5:554
pter-16.3	D4S125	6	1	0.17	Breast	CR 50:7184
16	D4S95	33	4	0.12	Breast	CR 53:4356
pter-16.3	D4S125	5	0	0	Cervix	CR 54:4481
Unknown	D4S125	2	0	0	Cervix	GCC 9:119
Unknown	D4S391	25	9	0.36	Cervix	CR 56:497
Unknown	D4S405	30	4	0.13	Cervix	CR 56:197
16	D4S10	11	0	0	Colon	CCG 48:167
pter-16.3	D4S125	8	0	0	Colon	CCG 48:167
11.0-15	D4S174	21	0	0	Endocrine	GCC 13:59
Unknown	D4S2397	18	1	0.06	Endocrine	CR 56:599
Unknown	D4S174	21	2	0.1	Esophageal	CR 54:2996
Unknown	D4S125	40	7	0.17	Esophageal	GCC 10:177
pter-16.3	D4S125	9	0	0	Esophageal	CR 51:2113
Unknown	D4S394	15	1	0.07	Head&Neck	CR 54:4756
Unknown	D4S394	18	0	0	Head&Neck	CR 54:4756
Unknown	D4S404	21	8	0.38	Head&Neck	CR 54:1152
pter-16.3	D4S125	7	0	0	Kidney	CR 51:820
Unknown	D4S431	28	2	0.07	Kidney	PNAS 92:2854
16.3	D4S10	5	1	0.2	Liver	CCG 48:72
16	D4S10	6	2	0.33	Liver	CR 51:4367
pter-16.3	D4S125	4	0	0	Liver	CR 51:89
Unknown	D4S125	6	0	0	Liver	PNAS 86:8852
16.1	RAF1P1	13	2	0.15	Liver	JJCR 81:108
pter-16.3	D4S125	28	2	0.07	Lung	CR 52:2478
pter-16.3	D4S125	24	10	0.42	Ovary	CR 51:5118
Unknown	D4S125-D4S124	29	10	0.34	Ovary	CR 53:2393
15.1-11	D4S16	19	2	0.11	Ovary	JJC 54:546
11.0-15	D4S174	20	3	0.15	Ovary	BJC 69:429
16.2-15.1	D4S49	20	5	0.25	Ovary	JJC 54:546
12.0-13	GABRB1	16	2	0.12	Ovary	BJC 69:429
pter-16.3	D4S125	3	0	0	Pancreas	CR 54:2761
12.0-13	GABRB1	13	0	0	Prostate	G 11:530
Unknown	D4S124	13	1	0.08	Sarcoma	CR 52:2419
Unknown	D4S125	17	3	0.18	Testis	O 9:2245
pter-16.3	D4S125	9	0	0	Testis	JU 73:606
Unknown	D4S129	10	1	0.1	Testis	GCC 13:249
pter-16.3	D4S125	2	0	0	Uterus	GCC 9:119
11.0-15	D4S174	21	1	0.05	Uterus	CR 54:4294
16	D4S43	25	1	0.04	Uterus	CR 54:4294
12.0-13	GABRB1	25	0	0	Uterus	CR 54:4294
16.1	RAF1P1	7	0	0	Uterus	CR 51:5632

Chromosome 4 - p Arm

SUM	729	93	0.13

Chromosome 4 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
p11-q21	MT2P1	4	0	0	Uterus	CR 51:5632
33-35	D4S171	29	15	0.52	Bladder	CR 55:5213
25-34	D4S249	29	15	0.52	Bladder	CR 55:5213
Unknown	Unknown	20	2	0.1	Brain	CR 50:5784
Unknown	D4S125	34	2	0.06	Breast	CR 50:7184
25-34	D4S192	54	13	0.24	Breast	BCRT 32:5
28	FGA	19	4	0.21	Breast	GCC 2:191
28	FGA	18	0	0	Breast	CR 53:4356
p11-q21	MT2P1	17	0	0	Breast	JNCI 84:506
21-23	ADH3	22	12	0.55	Cervix	CR 54:4481
21-25	ADH5	24	11	0.46	Cervix	CR 54:4481
Unknown	D4S163	41	12	0.29	Cervix	CR 54:4481
Unknown	D4S402	28	8	0.29	Cervix	CR 56:197
Unknown	D4S415	26	8	0.31	Cervix	CR 56:197
q11-q13	ALB	11	0	0	Colon	CCG 48:167
Unknown	D4S415	19	1	0.05	Endocrine	CR 56:599
Unknown	D4S163	21	2	0.1	Esophageal	CR 54:2996
Unknown	D4S163	35	9	0.26	Esophageal	GCC 10:177
Unknown	D4S402	16	3	0.19	Head&Neck	CR 54:4756
Unknown	D4S402	20	1	0.05	Head&Neck	CR 54:4756
Unknown	D4S430	24	9	0.38	Head&Neck	CR 54:1152
Unknown	D4S163	23	2	0.09	Kidney	CR 51:820
Unknown	D4S426-D4S415	20	1	0.05	Kidney	PNAS 92:2854
Unknown	D4S426-D4S415	5	0	0	Kidney	PNAS 92:2854
Unknown	D4S408-429	23	4	0.17	Leukemia	CR 55:5377
Unknown	Unknown	8	0	0	Liver	BJC 64:1083
21-23	ADH3	4	0	0	Liver	JJCR 81:108
21-23	ADH3	6	1	0.17	Liver	CR 51:4367
q11-q13	ALB	5	5	1	Liver	PNAS 86:8852
Unknown	D4S16	5	2	0.4	Liver	JJCR 81:108
Unknown	D4S163	20	3	0.15	Liver	CR 51:89
p11-q21	MT2P1	16	8	0.5	Liver	JJCR 81:108
p11-q21	MT2P1	21	9	0.43	Liver	JJCR 84:893
p11-q21	MT2P1	19	4	0.21	Liver	CR 54:281
Unknown	D4S163	31	8	0.26	Lung	CR 52:2478
21-23	ADH3	18	1	0.06	Ovary	IJC 54:546
11.0-15	D4S154D	20	3	0.15	Ovary	BJC 69:429
11.0-15	D4S1607	20	3	0.15	Ovary	BJC 69:429
Unknown	D4S163	16	1	0.06	Ovary	CR 51:5118
33-35	D4S171	12	4	0.33	Ovary	BJC 69:429
25-34	D4S175	20	7	0.35	Ovary	BJC 69:429
Unknown	D4S27	29	10	0.34	Ovary	CR 53:2393
p11-q21	MT2P1	21	2	0.1	Ovary	IJC 54:546
35	Unknown	6	1	0.17	Pancreas	CR 54:2761
28	FGA	9	0	0	Prostate	Q 11:530
Unknown	D4S163	17	3	0.18	Sarcoma	CR 52:2419

Chromosome 4 - q Arm

21-23	ADH3	24	0	0	Testis	O 9:2245
33-35	D4S171	23	0	0	Uterus	CR 54:4294
p11-q21	MT2P1	1	0	0	Uterus	CR 51:5632
SUM		952	209	0.22		

Chromosome 5 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D5S392	34	8	0.24	Cervix	JNCI 87:742
Unknown	D5S392	19	0	0	Endocrine	CR 56:599
Unknown	D5S392	26	5	0.19	Head&Neck	CR 54:1152
Unknown	D5S392	19	0	0	Kidney	PNAS 92:2854
Unknown	D5S392	5	0	0	Kidney	PNAS 92:2854
Unknown	D5S13	21	1	0.05	Breast	CR 53:4356
Unknown	D5S13	17	4	0.24	Breast	GCC 2:191
pter-p15	D5S4	10	1	0.1	Breast	GCC 2:191
pter-p15	D5S4	17	2	0.12	Colon	IJC 53:382
pter-p15	D5S4	11	0	0	Colon	CCG 48:167
pter-p15	D5S4	29	11	0.63	Colon	CR 50:7166
pter-p15	D5S4	19	4	0.21	Ovary	CR 53:2393
pter-p15	D5S4	3	0	0	Testis	CCG 52:72
pter-p15	D5S4	1	0	0	Testis	CCG 52:72
pter-p15	D5S4	1	0	0	Testis	CCG 52:72
15.1-15.2	D5S406	25	12	0.48	Cervix	JNCI 87:742
15.2-15.1	D5S12	12	1	0.08	Brain	CR 50:5784
15.2-15.1	D5S12	13	5	0.38	Cervix	CR 54:4481
15.2-15.1	D5S12	9	0	0	Ovary	O 5:219
15.2-15.1	D5S12	17	0	0	Prostate	G 11:530
15.2-15.1	D5S12	26	11	0.42	Testis	O 9:2215
15.1-15.3	D5S208	20	10	0.5	Cervix	JNCI 87:742
15-21	D5S630	5	2	0.4	Lung	O 12:97
15-21	D5S630	13	3	0.23	Lung	O 12:97
14	D5S432	29	8	0.28	Cervix	JNCI 87:742
15.1-15.3	D5S117	25	8	0.32	Cervix	JNCI 87:742
15.1-15.3	D5S117	13	2	0.15	Ovary	BJC 69:429
15.1-15.3	D5S117	22	1	0.05	Uterus	CR 54:4294
Unknown	D5S268	14	3	0.21	Ovary	BJC 69:429
Unknown	D5S419	26	3	0.12	Cervix	CR 56:197
Unknown	D5S419	28	0	0	Head&Neck	CR 54:4756
Unknown	D5S419	16	3	0.19	Head&Neck	CR 54:4756
14	D5S19	23	13	0.57	Cervix	CR 54:4481
Unknown	D5S395	28	6	0.21	Cervix	CR 56:197
13	D5S20	21	1	0.05	Ovary	IJC 54:546
11.0-13	D5S21	9	5	0.56	Cervix	CR 54:4481
11.0-13	D5S21	9	5	0.56	Cervix	CR 54:4481
Unknown	Unknown	4	0	0	Brain	CR 49:6572
Unknown	D5S1	5	1	0.2	Breast	GCC 2:191
Unknown	Unknown	5	0	0	Colon	BJC 67:1007
Unknown	D5S1	3	0	0	Colon	CCG 48:167
Unknown	D5S1	28	7	0.25	Esophageal	CR 54:2996
Unknown	Unknown	11	0	0	Liver	BJC 67:1007
Unknown	Unknown	8	3	0.38	Liver	BJC 64:1083
Unknown	Unknown	3	0	0	Pancreas	CR 54:2761
Unknown	Unknown	7	0	0	Pancreas	BJC 65:809

Chromosome 5 - p Arm

Unknown	Unknown	29	1	0.03	Testis	GCC 13:249
SUM		722	135	0.19		

Chromosome 5 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
15-21	D5S491	1	0	0	Lung	O 12:97
15-21	D5S491	8	3	0.38	Lung	O 12:97
Unknown	D5S427	22	4	0.18	Cervix	CR 56:197
11.2-13.3	D5S6	30	1	0.03	Breast	GE 5:554
11.2-13.3	D5S6	4	2	0.5	Colon	O 9:991
11.2-13.3	D5S6	32	9	0.28	Colon	CR 50:7166
11.2-13.3	D5S6	17	1	0.06	Pediatric	CR 50:3279
15-21	D5S637	5	1	0.2	Lung	O 12:97
15-21	D5S637	9	6	0.67	Lung	O 12:97
15-21	D5S626	4	1	0.25	Lung	O 12:97
15-21	D5S626	17	9	0.53	Lung	O 12:97
Unknown	D5S107	19	2	0.11	Leukemia	B 83:3449
Unknown	D5S107	33	2	0.06	Stomach	CR 56:612
Unknown	D5S107	30	1	0.03	Uterus	CR 54:4294
Unknown	D5S428	20	7	0.35	Stomach	CR 56:612
Unknown	D5S37	2	0	0	Colon	O 9:991
Unknown	D5S37	11	6	0.55	Colon	CR 50:7166
Unknown	D5S37	28	7	0.25	Esophageal	CR 54:2996
Unknown	D5S37	3	0	0	Liver	CCG 48:172
Unknown	D5S37	12	5	0.42	Sarcoma	CR 52:2419
Unknown	D5S37	18	4	0.22	Testis	GCC 13:249
15-21	D5S644	9	3	0.33	Lung	O 12:97
15-21	D5S644	22	12	0.55	Lung	O 12:97
14-21	D5S71	10	1	0.1	Colon	S 241:961
14-21	D5S71	6	3	0.5	Colon	CR 50:7166
14-21	D5S71	8	3	0.38	Colon	GCC 3:468
14-21	D5S71	4	0	0	Colon	CCG 48:167
14-21	D5S71	21	1	0.05	Ovary	IJC 54:546
14-21	D5S71	1	1	1	Pancreas	GCC 3:468
14-21	D5S71	6	0	0	Stomach	GCC 3:468
14-21	D5S71	6	2	0.33	Testis	GCC 13:249
14-21	D5S71	1	0	0	Uterus	CR 51:5632
Unknown	D5S409	17	1	0.06	Endocrine	CR 56:599
Unknown	D5S409	17	6	0.35	Stomach	CR 56:612
Unknown	D5S409	9	6	0.67	Stomach	CR 55:1933
14-21	D5S82	15	4	0.27	Colon	JJCR 82:10
Unknown	D5S82	16	1	0.06	Stomach	CR 54:41
21	D5S421	25	5	0.2	Bladder	CR 55:5213
21	D5S421	20	5	0.25	Head&Neck	CR 54:1152
21	D5S421	5	0	0	Kidney	GCC 12:76
21-22	D5S81	13	0	0.23	Cervix	BJC 67:71
Unknown	D5S81	31	19	0.61	Colon	CR 50:7166
21-22	D5S81	5	4	0.8	Colon	BJC 67:100
21-22	D5S81	18	4	0.22	Colon	JJCR 82:10
Unknown	D5S81	28	5	0.18	Kidney	CR 51:5817
21-22	D5S81	13	3	0.23	Kidney	CR 51:820

Chromosome 5 - q Arm

21-22	D5S81	6	1	0.17	Liver	BJC 64:108
21-22	D5S81	4	0	0	Liver	BJC 67:100
21-22	D5S81	5	1	0.2	Pancreas	BJC 65:809
21-22	D5S81	12	5	0.42	Stomach	HG 92:244
Unknown	D5S81	9	2	0.22	Testis	GCC 13:249
Unknown	L5.71	13	5	0.38	Colon	JJCR 82:10
Unknown	MCC	13	5	0.38	Colon	JJCR 82:10
21	MCC	4	1	0.25	Colon	O 9:991
21	MCC	31	9	0.29	Colon	CR 52:741
21	MCC	34	12	0.35	Colon	EJC 30A:66
21	MCC	35	22	0.63	Esophageal	CR 52:6525
Unknown	L5.71	2	2	1	Lung	CR 52:2478
Unknown	L5.71	16	4	0.25	Lung	CR 52:2478
Unknown	L5.71	1	1	1	Lung	CR 52:2478
Unknown	L5.71	4	0	0	Lung	CR 52:2478
Unknown	MCC	2	2	1	Lung	CR 52:2478
21	MCC	41	9	0.22	Lung	CR 55:220
Unknown	MCC	1	1	1	Lung	CR 52:2478
Unknown	MCC	16	4	0.25	Lung	CR 52:2478
Unknown	MCC	4	0	0	Lung	CR 52:2478
21	MCC	7	7	1	Stomach	JJCR 84:10
21	MCC	36	4	0.11	Stomach	CL 96:169
21	MCC	8	0	0	Stomach	CR 54:41
21	MCC-APC	25	7	0.28	Breast	BJC 68:64
21	MCC-APC	6	0	0	Cervix	GCC 9:119
21	MCC-APC	45	16	0.36	Colon	GAST 104:1
21	MCC-APC	56	37	0.66	Colon	O 8:1391
21	MCC-APC	26	20	0.77	Esophageal	PNAS 89:33
21	MCC-APC	6	4	0.67	Lung	CR 55:513
21	MCC-APC	5	2	0.4	Lung	CR 52:1996
21	MCC-APC	7	0	0	Uterus	GCC 9:119
21	APC	21	7	0.33	Colon	CR 52:741
Unknown	APC	37	3	0.08	Colon	EJC 30A:66
Unknown	APC	33	6	0.18	Colon	EJC 30A:66
21	APC	21	5	0.24	Esophageal	GCC 10:177
21	APC	36	24	0.67	Esophageal	CR 52:6525
21	APC	19	1	0.05	Liver	CR 54:281
21	APC	20	14	0.7	Lung	O 12:97
21	APC	53	17	0.32	Lung	CR 55:2203
21	APC	7	5	0.71	Lung	CR 54:1772
21	APC	8	3	0.38	Lung	O 12:97
Unknown	APC	18	9	0.5	Ovary	GO 55:245
Unknown	APC	15	3	0.2	Prostate	JU 151:1D7
21	APC	7	3	0.43	Prostate	BJU 73:390
Unknown	APC	13	4	0.31	Stomach	LT 24:835
Unknown	APC	35	3	0.09	Stomach	CL 96:169

Chromosome 5 - q Arm

21	APC	12	0	0	Stomach	CR 54:41
21	APC	14	12	0.86	Stomach	JJCR 84:10
21-22	D5S346	38	0	0	Endocrine	GCC 13:9
21-22	D5S346	46	1	0.02	Kidney	BJC 69:230
21-22	D5S346	15	6	0.4	Ovary	BJC 69:429
21-22	D5S346	18	2	0.11	Stomach	CR 56:612
21-22	D5S346	22	1	0.05	Uterus	CR 54:4294
Unknown	Unknown	19	3	0.16	Colon	JJCR 82:10
Unknown	Unknown	10	2	0.2	Kidney	CR 51:5817
21-22	D5S84	11	2	0.18	Breast	CR 50:7184
21-22	D5S84	21	1	0.05	Breast	CR 53:4556
21-22	D5S84	3	1	0.33	Cervix	GCC 9:119
21-22	D5S84	8	0	0	Cervix	BJC 67:91
21-22	D5S84	5	2	0.4	Kidney	CR 51:5817
21-22	D5S84	5	2	0.4	Kidney	CR 51:5820
21-22	D5S84	9	4	0.44	Liver	CR 51:89
21-22	D5S84	15	0	0	Ovary	CR 51:5118
21-22	D5S84	13	1	0.08	Uterus	GCC 9:119
21-22	D5S86	6	2	0.33	Colon	GCC 3:468
21-22	D5S86	4	1	0.25	Pancreas	GCC 3:468
21-22	D5S86	8	3	0.38	Stomach	GCC 3:468
31-33	D5S804	19	6	0.32	Ovary	GO 55:245
21-22	FBN2	15	6	0.4	Ovary	BJC 69:429
21-22	FBN2	15	4	0.27	Stomach	CR 56:612
33-35	D5S70	24	9	0.38	Cervix	CR 54:4481
33-35	D5S70	3	0	0	Colon	GCC 3:468
33-35	D5S70	3	0	0	Pancreas	GCC 3:468
33-35	D5S70	13	5	0.38	Stomach	GCC 3:468
33-35	D5S70	13	3	0.23	Testis	D 9:2245
21-22	D5S178	15	6	0.4	Ovary	BJC 69:429
21-22	D5S178	19	2	0.11	Stomach	CR 56:612
31-32	GRL	8	0	0	Ovary	CR 50:2724
21-22	D5S210	15	6	0.4	Ovary	BJC 69:429
21-22	D5S210	19	5	0.26	Stomach	CR 56:612
21-22	D5S209	15	6	0.4	Ovary	BJC 69:429
21-22	D5S209	23	2	0.09	Stomach	CR 56:612
34-qter	D5S22	18	0	0	Prostate	G 11:530
34-qter	D5S2	3	1	0.33	Cervix	CR 49:3598
34-qter	D5S2	2	0	0	Colon	N 331:275
34-qter	D5S2	8	0	0	Liver	JJCR 81:10
34-qter	D5S2	11	1	0.09	Lung	PN 84:9252
Unknown	D5S2	11	1	0.09	Lung	PNAS 84:92
Unknown	D5S2	5	1	0.2	Stomach	CR 52:3099
34-qter	D5S2	2	0	0	Stomach	CR 48:2988
34-qter	D5S2	1	0	0	Uterus	CR 51:5632
Unknown	D5S400	32	5	0.16	Cervix	CR 56:197

Chromosome 5 - q Arm

Unknown	D5S429	3	0	0	Kidney	PNAS 92:28
Unknown	D5S429	19	1	0.05	Kidney	PNAS 92:28
35-qter	D5S43	17	1	0.06	Colon	CR 50:7166
35-qter	D5S43	5	2	0.4	Colon	BJC 67:100
35-qter	D5S43	31	9	0.29	Colon	BJC 59:750
35-qter	D5S43	10	0	0	Endocrine	N 328:524
35-qter	D5S43	10	3	0.3	Liver	BJC 67:100
35-qter	D5S43	10	5	0.5	Liver	BJC 64:108
35-qter	D5S43	7	0	0	Pancreas	CR 54:2761
35-qter	D5S43	11	0	0	Pancreas	BJC 65:809
35-qter	D5S43	10	1	0.1	Stomach	BJC 59:750
35-qter	D5S43	34	8	0.24	Stomach	CR 51:2926
35-qter	D5S43	25	5	0.2	Testis	GCC 13:249
35-qter	D5S43	25	5	0.2	Testis	GCC 13:249
Unknown	Unknown	12	2	0.17	Brain	CR 50:5784
15-21	Unknown	6	0	0	Cervix	BJC 67:71
21	Unknown	2	0	0	Cervix	BJC 67:71
Unknown	Unknown	2	1	0.5	Cervix	BJC 67:71
Unknown	Unknown	11	2	0.18	Cervix	BJC 67:71
Unknown	Unknown	23	8	0.35	Colon	JJCR 82:10
Unknown	Unknown	2	1	0.5	Colon	JJCR 82:10
Unknown	Unknown	19	7	0.37	Colon	JJCR 82:10
Unknown	Unknown	1	1	1	Colon	JJCR 82:10
Unknown	Unknown	17	1	0.06	Colon	JJCR 82:10
Unknown	Unknown	10	5	0.5	Colon	JJCR 82:10
Unknown	Unknown	17	6	0.35	Colon	JJCR 82:10
Unknown	Unknown	31	0	0	Colon	JJCR 82:10
15-21	Unknown	1	1	1	Colon	BJC 67:100
21	Unknown	4	3	0.75	Colon	BJC 67:100
21	C11p11	3	1	0.33	Colon	N 331:273
Unknown	CRI-L1265	16	1	0.06	Colon	S 241:961
Unknown	CRI-L45	21	2	0.1	Colon	S 241:961
33	C9F1R	11	4	0.36	Colon	CR 50:7166
21	D5S141	3	2	0.67	Colon	BJC 67:100
Unknown	FMS	9	2	0.22	Colon	N 331:273
21-22	LS5.34	5	3	0.6	Colon	CR 50:7166
21	D5S141	35	13	0.37	Esophageal	GCC 10:177
Unknown	D5S410	31	1	0.03	Head&Neck	CR 54:4756
Unknown	D5S410	35	4	0.11	Head&Neck	CR 54:4756
21	D5S133	6	1	0.17	Kidney	CR 51:5817
21	D5S140	16	3	0.19	Kidney	CR 51:5817
21	D5S141	26	8	0.31	Kidney	CR 51:5817
Unknown	D5S89	15	5	0.33	Leukemia	B 83:799
Unknown	Unknown	10	1	0.1	Liver	CR 51:89
21	Unknown	6	0	0	Liver	BJC 67:100
15-21	Unknown	5	0	0	Liver	BJC 67:100

Chromosome 5 - q Arm

21	D5S141	7	0	0	Liver	BJC 67:100
21-21-34-qter	D5S43-D5S81	45	14	0.31	Liver	JJCR 84:89
21	ECB27	8	1	0.12	Liver	BJC 64:108
Unknown	FMS	2	0	0	Lung	PN 84:9252
13-12	del-27	15	11	0.73	Lung	O 12:97
13-12	del-27	8	3	0.38	Lung	O 12:97
13-12	del-27	7	4	0.57	Lung	CR 54:1772
21	D5S122	11	5	0.45	Ovary	GO 55:245
Unknown	D5S6-D5S107-APC	37	16	0.43	Ovary	CR 53:2393
21-22	IRF-1	15	6	0.4	Ovary	BJC 69:429
15-21	Unknown	6	0	0	Pancreas	BJC 65:809
15-21	D5S98	13	3	0.23	Stomach	HG 92:244
21-22	IRF-1	22	6	0.27	Stomach	CR 56:612
15-21	D5S98	7	1	0.14	Testis	GCC 13:249
Unknown	FMS	21	1	0.05	Uterus	CR 54:4294
SUM		2866	763	0.27		

Chromosome 6 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D6S477	33	15	0.45	Colon	CR 56:145
24-25	F13A1	18	5	0.28	Ovary	GO 55:245
24-25	F13A1	18	4	0.22	Ovary	BJC 69:429
Unknown	D6S309	18	1	0.06	Kidney	PNAS 92:2854
Unknown	D6S309	4	1	0.25	Kidney	PNAS 92:2854
pter-p25	D6F21S1	12	4	0.33	Ovary	BJC 67:551
Unknown	D6S89	14	1	0.07	Ovary	BJC 67:551
Unknown	D6S289	36	13	0.36	Colon	CR 56:145
Unknown	D6S260	32	14	0.44	Cervix	CR 56:197
21.3-24	D6S109	17	3	0.18	Ovary	BJC 69:429
21.3-24	D6S109	16	2	0.12	Uterus	CR 54:4294
Unknown	D6S276	20	10	0.5	Cervix	CR 56:197
Unknown	D6S299	21	1	0.05	Head&Neck	CR 54:4756
Unknown	D6S299	20	0	0	Head&Neck	CR 54:4756
Unknown	D6S299	26	2	0.08	Melanoma	CR 56:589
Unknown	D6S105	27	2	0.07	Esophageal	IJC 69:1
Unknown	D6S105	19	4	0.21	Head&Neck	CR 54:1152
Unknown	D6S105	26	2	0.08	Uterus	CR 54:4294
Unknown	D6S258	33	15	0.45	Colon	CR 56:145
Unknown	D6S10	35	4	0.11	Breast	GCC 2:191
Unknown	D6S10	32	9	0.28	Cervix	CR 54:4481
Unknown	D6S10	2	0	0	Pancreas	CR 54:2761
Unknown	D6S10	13	0	0	Prostate	G 11:530
Unknown	D6S10	32	4	0.12	Testis	O 9:2245
21.3	HLA-DRB	21	3	0.14	Ovary	BJC 67:551
21.3	HLA-DQA	18	4	0.22	Ovary	BJC 67:551
21.3	HLA-DQA	3	0	0	Testis	CCG 52:72
21.3	HLA-DQA	1	0	0	Testis	CCG 52:72
21.3	HLA-DQA	4	0	0	Testis	CCG 52:72
Unknown	TNFa	33	14	0.42	Colon	CR 56:145
Unknown	D6S291	12	1	0.08	Brain	CR 55:4696
Unknown	D6S291	12	1	0.08	Brain	CR 55:4696
Unknown	D6S29	17	0	0	Colon	CCG 48:167
Unknown	D6S29	22	3	0.14	Kidney	CR 51:5817
Unknown	D6S29	13	1	0.08	Liver	CR 51:89
Unknown	D6S29	12	6	0.5	Ovary	CR 51:5118
Unknown	D6S29	19	4	0.21	Ovary	IJC 54:546
Unknown	D6S29	9	0	0	Ovary	CR 50:2724
Unknown	D6S29	16	3	0.19	Stomach	GCC 14:28
Unknown	D6S271	44	17	0.39	Colon	CR 56:145
Unknown	D6S282	32	6	0.19	Cervix	CR 56:197
Unknown	D6S282	22	0	0	Endocrine	CR 56:599
12.0-11	KRAS P1	8	1	0.12	Ovary	BJC 67:551
12.0-11	KRAS P1	2	0	0	Uterus	CR 51:5632
11.2	D6S291	37	11	0.3	Ovary	GCC 15:223
Unknown	D6S257	42	13	0.31	Colon	CR 56:145

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Unknown	D6S257	42	13	0.31	Colon	CR 56:145
Unknown	Unknown	14	1	0.07	Brain	CR 50:5783
Unknown	D6S40	24	2	0.08	Brain	CR 49:6573
Unknown	D6S40	28	5	0.18	Breast	CR 50:7184
Unknown	D6S40	3	1	0.33	Cervix	GCC 9:119
Unknown	D6S344	22	0	0	Endocrine	CR 56:599
Unknown	D6S109	40	12	0.24	Esophageal	GCC 10:177
Unknown	D6S40	23	7	0.3	Esophageal	CR 54:2996
Unknown	D6S40	14	1	0.07	Esophageal	CR 51:2114
Unknown	D6S265	19	8	0.42	Head&Neck	CR 54:1152
Unknown	TCTE	14	2	0.14	Head&Neck	CR 54:1152
21.3	D6S138	34	6	0.18	Kidney	CR 51:5817
21.2	D6S160	23	5	0.22	Kidney	CR 51:5817
Unknown	D6S4-C2-D6S1	19	5	0.26	Kidney	CR 49:5087
Unknown	D6S40	14	3	0.21	Kidney	CR 51:920
Unknown	Unknown	20	15	0.75	Lung	CR 54:2322
Unknown	D6S4-C2-D6S1	1	1	1	Lung	CR 49:5087
Unknown	D6S40	22	4	0.18	Lung	CR 52:2478
24-27	Unknown	7	2	0.29	Ovary	O 5:219
Unknown	D6S114E	3	0	0	Ovary	BJC 67:551
Unknown	D6S40	7	4	0.57	Ovary	O 5:219
Unknown	F13A1- D6S249	17	4	0.24	Ovary	BJC 72:1330
12-21.3	FTHP1	14	5	0.36	Ovary	BJC 69:429
12-21.2	FTHP1	10	2	0.2	Ovary	BJC 67:551
Unknown	PIM-HLA-D6S91-D6S41	34	21	0.62	Ovary	CR 53:2391
Unknown	D6S4-C2-D6S1	2	1	0.5	Sarcoma	CR 49:5087
Unknown	D6S40	13	7	0.54	Sarcoma	CR 52:2419
21.3	HLA-DXA	2	0	0	Testis	CCG 52:72
21.3	HLA-DXA	2	0	0	Testis	CCG 52:72
21.3	HLA-DXA	1	0	0	Testis	CCG 52:72
Unknown	D6S40	5	0	0	Uterus	GCC 9:119
SUM		1383	328	0.24		

Chromosome 6 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D6Z1	8	2	0.25	Ovary	BJC 67:551
Unknown	D6Z1	22	0	0	Stomach	GCC 14:28
13	D6S313	30	3	0.1	Breast	BJC 71:290
13	D6S254	5	0	0	Breast	BJC 73:144
13	D6S280	20	8	0.4	Breast	BJC 71:290
14-15	D6S284	26	5	0.19	Breast	BJC 71:290
14-15	D9S284	5	1	0.2	Breast	BJC 73:144
16.3-21	D6S286	27	8	0.3	Breast	BJC 71:290
14-15	D6S286	11	4	0.36	Breast	BJC 73:144
16.3-21	D6S286	17	1	0.06	Endocrine	CR 56:599
14-15	D69286	17	8	0.47	Ovary	GCC 15:223
Unknown	EDDR1	14	4	0.29	Ovary	GCC 15:223
22.3-23.1	D6S270	5	1	0.2	Breast	BJC 73:144
22.3-23.1	D6S270	22	7	0.32	Ovary	GCC 15:223
Unknown	D6S310	23	7	0.3	Endocrine	CR 56:599
Unknown	D6S310	33	10	0.3	Ovary	GCC 15:223
Unknown	D6S311	27	5	0.19	Cervix	CR 56:197
Unknown	D6S311	6	4	0.67	Endocrine	CR 56:599
Unknown	D6S311	32	10	0.31	Ovary	GCC 15:223
Unknown	D6S194	4	0	0	Ovary	CR 52:5815
Unknown	D6S194	16	5	0.31	Ovary	GCC 15:223
Unknown	D6S194	16	4	0.25	Ovary	CR 52:5815
Unknown	D6S142	30	8	0.27	Kidney	CR 51:5817
Unknown	D6S142	6	0	0	Ovary	CR 52:5815
Unknown	D6S142	12	7	0.58	Ovary	CR 52:5815
Unknown	D6S142	6	0	0	Ovary	CR 52:5815
Unknown	D6S161	27	6	0.22	Kidney	CR 51:5817
Unknown	D6S161	11	0	0	Ovary	CR 52:5815
Unknown	D6S161	17	7	0.41	Ovary	CR 52:5815
Unknown	D6S161	5	1	0.2	Ovary	CR 52:5815
Unknown	D6S251	67	16	0.24	Breast	BJC 73:144
Unknown	D6S251	36	13	0.36	Colon	CR 56:145
Unknown	D6S251	15	0	0	Ovary	CR 55:2169
Unknown	D6S251	28	0	0	Ovary	CR 55:2169
13	D6S239	27	9	0.33	Breast	BJC 71:290
13	D6S239	10	3	0.3	Ovary	CR 55:2169
13	D6S239	27	1	0.04	Ovary	CR 55:2169
14-16.2	D6S252	48	11	0.23	Breast	BJC 73:144
14-16.2	D6S252	27	2	0.07	Stomach	GCC 14:28
14	D6S300	32	11	0.34	Breast	BJC 71:290
14	D6S300	17	3	0.18	Endocrine	CR 56:599
16.3	D6S246	27	9	0.33	Breast	BJC 71:290
Unknown	D6S246	16	1	0.06	Ovary	CR 55:2169
Unknown	D6S246	9	2	0.22	Ovary	CR 55:2169
16.3-21	D6S249	28	9	0.32	Breast	BJC 73:144
16.3-21	D6S283	30	5	0.17	Breast	BJC 71:290

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16.3-21	D69283	10	2	0.2	Stomach	GCC 14:28
Unknown	D6S268	4	1	0.25	Kidney	GCC 12:76
Unknown	D6S268	9	1	0.11	Stomach	GCC 14:28
16.3-21	D6S302	30	13	0.43	Breast	BJC 73:144
21-23.3	D6S261	34	7	0.21	Breast	BJC 71:290
21-23	D6S261	25	5	0.2	Breast	BJC 73:144
21-23	D6S287	33	4	0.12	Breast	BJC 73:144
21-23	D6S287	22	4	0.18	Endocrine	CR 56:599
Unknown	D69267	18	5	0.28	Ovary	GCC 15:223
22.3-23.1	ARG	12	2	0.17	Breast	BJC 73:144
22.3-23.1	ARG	15	0	0	Stomach	GCC 14:28
22.3-23.1	D6S262	28	10	0.36	Breast	BJC 73:144
Unknown	D6S262	35	12	0.34	Colon	CR 56:145
Unknown	D6S262	17	1	0.06	Head&Neck	CR 54:4756
Unknown	D6S262	21	3	0.14	Head&Neck	CR 54:4756
Unknown	D6S32	18	9	0.5	Stomach	GCC 14:28
23.1	D6S87	17	6	0.35	Ovary	BJC 69:479
23.1	D6S87	18	3	0.17	Ovary	CR 55:2169
23.1	D6S87	7	2	0.29	Ovary	CR 55:2169
23.1	D6S87	20	1	0.05	Uterus	CR 54:4294
22-23	MYB	10	0	0	Cervix	CR 49:3598
22-23	MYB	11	2	0.18	Colon	N 331:273
22-23	MYB	20	2	0.1	Colon	IJC 53:302
22-23	MYB	13	0	0	Liver	JJCR 81:108
22-23	MYB	18	3	0.17	Lung	PN 84:9252
22-23	MYB	7	3	0.43	Melanoma	CR 51:5449
22-23	MYB	5	0	0	Neuroblastoma	CR 49:1095
22-23	MYB	9	6	0.67	Ovary	BJC 67:551
22-23	MYB	4	1	0.25	Ovary	GO 55:245
22-23	MYB	8	1	0.12	Ovary	CR 50:2724
22-23	MYB	7	0	0	Prostate	G 11:530
22-23	MYB	20	6	0.3	Sarcoma	CR 52:2419
22-23	MYB	12	1	0.08	Stomach	GCC 14:28
22-23	MYB	13	0	0	Stomach	CR 48:2988
22-23	MYB	12	2	0.17	Stomach	CR 52:3099
22-23	MYB	7	1	0.14	Uterus	CR 51:5632
Unknown	D6S250	24	1	0.04	Ovary	CR 55:2169
Unknown	D6S250	10	3	0.3	Ovary	CR 55:2169
Unknown	D69136	16	2	0.12	Kidney	CR 51:5817
Unknown	D6S136	3	0	0	Ovary	CR 52:5815
Unknown	D6S136	9	0	0	Ovary	CR 52:5815
Unknown	D6S441	11	1	0.09	Endocrine	CR 56:599
Unknown	D69441	30	13	0.43	Ovary	GCC 15:223
24-27	ESR	16	0	0	Cervix	CGC 79:74
24-27	ESR	8	3	0.38	Colon	GCC 3:1468
24-27	ESR	8	4	0.5	Melanoma	CR 51:5449

Chromosome 6 - q Arm

24-27	ESR	23	6	0.26	Ovary	CR 55:2169
24-27	ESR	6	1	0.17	Ovary	CR 55:2169
24-27	ESR	13	2	0.15	Ovary	GO 47:137
24-27	ESR	14	9	0.64	Ovary	CR 50:2724
24-27	ESR	22	1	0.05	Ovary	IJC 54:546
24-27	ESR	15	10	0.67	Ovary	BJC 67:551
24-27	ESR	18	10	0.56	Ovary	GCC 15:223
24-27	ESR	1	1	1	Pancreas	GCC 3:468
24-27	ESR	6	0	0	Stomach	GCC 3:468
24-27	ESR	16	0	0	Stomach	CR 51:2926
24-27	ESR	6	1	0.17	Uterus	CR 51:5632
Unknown	D6S415	22	9	0.41	Ovary	GCC 15:223
25.2	D6S255	9	3	0.33	Breast	BJC 73:1412
25.2	D6S255	23	2	0.09	Head&Neck	CR 54:1152
25.2	D6S255	7	3	0.43	Ovary	CR 55:2169
25.2	D6S255	11	2	0.18	Ovary	CR 55:2169
Unknown	D6S305	29	4	0.14	Cervix	CR 56:197
Unknown	D6S305	40	16	0.4	Colon	CR 56:145
Unknown	D6S305	15	2	0.13	Endocrine	CR 56:599
Unknown	D6S305	29	9	0.31	Melanoma	CR 56:589
Unknown	D6S305	35	11	0.37	Ovary	GCC 15:223
Unknown	IGF2R	16	11	0.69	Liver	O 10:1725
Unknown	IGF2R	2	0	0	Ovary	CR 55:2169
Unknown	IGF2R	4	1	0.25	Ovary	CR 55:2169
Unknown	IGF2R	18	5	0.28	Ovary	GCC 15:223
Unknown	IGF2R	11	3	0.27	Ovary	CR 55:2169
Unknown	IGF2R	7	0	0	Ovary	CR 55:2169
Unknown	IGF2R	18	2	0.11	Stomach	GCC 14:28
Unknown	IGF2R	10	2	0.2	Uterus	CR 54:4294
26-27	PLG	2	0	0	Liver	PNAS 86:8852
Unknown	D6S195	14	5	0.36	Ovary	CR 52:5815
Unknown	D6S195	2	0	0	Ovary	CR 52:5815
Unknown	D6S195	5	0	0	Ovary	CR 52:5815
Unknown	D6S191	16	3	0.19	Ovary	CR 52:5815
Unknown	D6S191	5	0	0	Ovary	CR 52:5815
Unknown	D6S191	8	0	0	Ovary	CR 52:5815
26	D6S186	25	5	0.2	Breast	BJC 71:290
26	D6S186	34	7	0.21	Kidney	CR 51:5817
26	D6S186	19	8	0.42	Ovary	CR 52:5815
26	D6S186	19	8	0.42	Ovary	GCC 15:223
26	D6S186	6	1	0.17	Ovary	CR 52:5815
26	D6S186	5	0	0	Ovary	CR 52:5815
Unknown	SOD2	11	3	0.27	Melanoma	CR 51:5449
Unknown	SOD2	8	4	0.5	Ovary	BJC 67:551
Unknown	SOD2	23	5	0.22	Stomach	GCC 14:26
Unknown	D6S264	32	13	0.41	Colon	CR 56:145

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Unknown	D6S264	12	5	0.42	Endocrine	CR 56:599
Unknown	D6S264	15	5	0.33	Head&Neck	CR 54:1152
Unknown	D6S264	3	1	0.33	Kidney	GCC 12:76
Unknown	D6S264	34	12	0.35	Ovary	GCC 15:223
Unknown	D6S303	34	14	0.41	Colon	CR 56:145
21-qter	D6S2	8	3	0.38	Colon	GCC 3:468
21-qter	D6S2	19	4	0.21	Ovary	IJC 52:575
21-qter	D6S2	5	3	0.6	Ovary	O 5:219
21-qter	D6S2	20	1	0.05	Ovary	IJC 54:546
21-qter	D6S2	1	1	1	Pancreas	GCC 3:468
21-qter	D6S2	6	0	0	Stomach	GCC 3:468
Unknown	D6S133	22	14	0.64	Ovary	BJC 67:551
Unknown	D6S193	56	9	0.16	Esophageal	GCC 10:177
Unknown	D6S193	38	23	0.61	Ovary	GCC 15:223
27	D6S297	19	4	0.21	Breast	BJC 71:290
Unknown	D6S297	27	14	0.52	Ovary	GCC 15:223
Unknown	TCP10	17	12	0.71	Ovary	BJC 67:551
27	D6S44	56	4	0.07	Breast	CR 53:4356
27	D6S44	12	4	0.33	Breast	GCC 7:191
27	D6S44	29	4	0.14	Ovary	IJC 54:546
27	D6S44	18	0	0	Testis	IJC 73:606
Unknown	D6S149	19	6	0.32	Ovary	GCC 15:223
Unknown	D6S149	8	2	0.25	Ovary	CR 52:5815
Unknown	D6S149	9	1	0.11	Ovary	CR 52:5815
Unknown	D6S149	22	10	0.45	Ovary	CR 52:5815
Unknown	D6S37	4	1	0.25	Breast	CR :53:3804
Unknown	D6S37	23	2	0.09	Breast	CR 50:7194
Unknown	D6S37	20	4	0.2	Cervix	CR 54:4481
Unknown	D6S37	5	2	0.4	Cervix	GCC 9:119
Unknown	D6S37	5	4	0.8	Endocrine	CR 56:599
Unknown	D6S37	13	2	0.15	Esophageal	CR 54:2996
Unknown	D6S37	13	4	0.31	Kidney	CR 51:820
Unknown	D6S37	25	9	0.36	Kidney	CR 51:5817
Unknown	D6S37	29	1	0.03	Lung	CR 52:2478
Unknown	D6S37	10	4	0.4	Melanoma	CR 51:5449
Unknown	D6S37	13	8	0.62	Ovary	BJC 67:551
Unknown	D6S37	29	5	0.17	Ovary	CR 51:5118
Unknown	D6S37	14	3	0.21	Sarcoma	CR 52:2419
Unknown	D6S37	30	11	0.37	Stomach	GCC 14:28
Unknown	D6S37	29	2	0.07	Testis	O 9:2245
Unknown	D6S37	11	1	0.09	Uterus	GCC 9:119
27	D6S446	24	11	0.46	Ovary	GCC 15:223
Unknown	D6S132	15	11	0.73	Ovary	BJC 67:551
27	D6S281	27	5	0.19	Breast	BJC 71:290
27	D6S281	39	13	0.33	Ovary	GCC 15:223
27	D6S281	39	13	0.33	Ovary	GCC 15:223

Chromosome 6 - q Arm

Unknown	Unknown	22	2	0.09	Brain	CR 50:5784
27	D6S193	29	8	0.28	Breast	BJC 71:290
25.2-27	D6S220	19	5	0.26	Breast	BJC 71:290
14-15	D6S330	12	6	0.5	Breast	BJC 71:290
23.3-25.2	D6S355	24	4	0.17	Breast	BJC 71:290
21-23.3	D6S357	20	2	0.1	Breast	BJC 71:290
21-23.3	D6S359	37	8	0.22	Breast	BJC 71:290
14-16	D6S39	1	1	1	Breast	CR 53:3804
16-21	D6S48	3	1	0.33	Breast	CR 53:3804
25.1	ER	47	9	0.19	Breast	BJC 71:448
24	D6S135	9	5	0.56	Kidney	CR 51:5817
21	D6S154	15	3	0.2	Kidney	CR 51:5817
27	D6S156	27	7	0.26	Kidney	CR 51:5817
23	D6S164	11	1	0.09	Kidney	CR 51:5817
Unknown	D6S281-D6S311-D6S278	22	4	0.18	Kidney	PNAS 92:2854
Unknown	D6S281-D6S311-D6S278	6	1	0.17	Kidney	PNAS 92:2854
Unknown	Unknown	20	15	0.75	Lung	CR 54:2322
12.0-21	CGA	13	3	0.23	Melanoma	CR 51:5449
Unknown	D6S29	4	0	0	Melanoma	CR 51:5449
27	Unknown	130	4	0.03	Ovary	IJC 52:575
Unknown	Unknown	23	1	0.04	Ovary	IJC 52:575
13	ACTBP2	21	7	0.33	Ovary	GO 55:245
Unknown	D6S175	17	4	0.24	Ovary	BJC 67:551
27	D6S193	10	1	0.1	Ovary	CR 52:5815
27	D6S193	11	1	0.09	Ovary	CR 52:5815
27	D6S193	23	11	0.48	Ovary	CR 52:5815
Unknown	D6S275	26	0	0	Ovary	CR 55:2169
Unknown	D6S225	13	2	0.15	Ovary	CR 55:2169
23.3-25.2	D6S355	6	0	0	Ovary	CR 55:2169
Unknown	D6S366	14	2	0.14	Ovary	CR 55:2169
Unknown	D6S366	19	1	0.05	Ovary	CR 55:2169
Unknown	D6S86	22	13	0.59	Ovary	BJC 67:551
Unknown	HCG-A	8	4	0.5	Ovary	BJC 67:551
Unknown	IGF2R-D6S:251-249	17	3	0.18	Ovary	BJC 72:1330
Unknown	MYB-DMDL-SOD2-D6S14	37	21	0.57	Ovary	CR 53:2393
27	Unknown	3	0	0	Pancreas	CR 54:2761
21.3	TNFB	13	2	0.15	Uterus	CR 54:4294
SUM		3960	978	0.25		

Chromosome 7 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
22	D7S21	36	5	0.14	Stomach	CR 51:2926
22	D7S21	19	1	0.05	Stomach	HG 92:244
22	D7S21	26	1	0.04	Testis	GCC 13:240
Unknown	D7S517	6	0	0	Kidney	PNAS 92:2854
Unknown	D7S517	21	0	0	Kidney	PNAS 92:2854
Unknown	D7S370	18	3	0.17	Brain	CR 50:5784
Unknown	D7S370	8	1	0.12	Breast	CR 50:7184
Unknown	D7S370	24	2	0.08	Cervix	CR 54:4481
Unknown	D7S370	24	5	0.21	Esophageal	CR 54:4956
Unknown	D7S370	10	2	0.2	Kidney	CR 51:820
Unknown	D7S370	10	0	0	Liver	CR 51:89
Unknown	D7S370	18	5	0.28	Lung	CR 52:2478
Unknown	D7S370	26	4	0.15	Ovary	BJC 73:1630
Unknown	D7S370	2	2	1	Pancreas	CR 54:2761
Unknown	D7S370	21	1	0.04	Testis	CR 52:2245
Unknown	D7S370	20	2	0.1	Esophageal	GCC 10:177
Unknown	D7S370	10	1	0.1	Esophageal	CR 51:2419
Unknown	D7S370	7	3	0.43	Ovary	CR 51:5118
Unknown	D7S370	17	2	0.12	Sarcoma	CR 52:2419
Unknown	D7S371	21	1	0.05	Breast	CR 53:4356
Unknown	D7S371	2	0	0	Ovary	CR 51:5118
13.0-12	EGFR	8	1	0.12	Cervix	CR 49:3598
13.0-12	EGFR	1	0	0	Liver	PNAS 86:8852
11.2-12	EGFR	18	3	0.17	Ovary	BJC 69:429
11.2-12	EGFR	14	0	0	Ovary	CR 49:1720
13.0-12	EGFR	5	1	0.2	Ovary	CR 50:2724
Unknown	EGFR	11	0	0	Ovary	CR 50:2724
13.0-12	EGFR	13	1	0.08	Prostate	G 11:530
Unknown	EGFR	10	0	0	Uterus	CR 51:5632
13.0-12	EGFR	16	2	0.12	Uterus	CR 54:4294
13.0-12	EGFR	16	2	0.12	Uterus	CR 54:4294
Unknown	D7S372	12	0	0	Brain	CR 49:6572
Unknown	D7S493	32	2	0.06	Cervix	CR 56:197
Unknown	D7S507	25	1	0.04	Cervix	CR 56:197
2.2-ter	Unknown	85	1	0.01	Colon	BJC 59:750
Unknown	D7S481	22	16	0.73	Colon	CR 56:145
Unknown	D7S507	20	1	0.05	Endocrine	CR 56:599
Unknown	D7S481	21	0	0	Head&Neck	CR 54:4756
Unknown	D7S481	22	4	0.18	Head&Neck	CR 54:4756
Unknown	D7S507	26	6	0.23	Head&Neck	CR 54:1152
pter-q22	Unknown	11	1	0.09	Liver	BJC 64:1089
pter-q22	Unknown	13	1	0.08	Liver	BJC 67:1007
Unknown	D7S481	36	2	0.05	Melanoma	CR 56:589
Unknown	D7S135	11	4	0.36	Ovary	CR 53:2393
pter-q22	Unknown	10	0	0	Pancreas	BJC 65:809
2.2-ter	Unknown	10	0	0	Stomach	BJC 59:750

Chromosome 7 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
21.3-22.1	COLIA2	29	1	0.03	Breast	GCC 2:191
21.3-22.1	COLIA2	6	0	0	Cervix	CR 49:3598
21.3-22.1	COLIA2	12	0	0	Colon	N 331:274
21.3-22.1	COLIA2	15	1	0.07	Liver	JJCR 81:108
21.3-22.1	COLIA2	11	0	0	Liver	CCG 48:72
21.3-22.1	COLIA2	5	0	0	Neuroblastoma	CR 49:1095
21.3-22.1	COLIA2	10	2	0.2	Stomach	CR 52:3099
21.3-22.1	COLIA2	6	0	0	Uterus	CR 51:5632
Unknown	D7S527	21	4	0.19	Breast	PNAS 91:12155
Unknown	D7S527	8	1	0.12	Colon	CR 55:1347
Unknown	D7S527	9	2	0.22	Head&Neck	CR 55:1347
Unknown	D7S527	8	1	0.12	Prostate	CR 54:6370
Unknown	D7S479	12	1	0.08	Breast	PNAS 91:12155
Unknown	D7S479	17	0	0	Endocrine	CR 56:599
Unknown	D7S518	27	6	0.22	Breast	PNAS 91:12155
Unknown	D7S518	8	0	0	Colon	CR 55:1347
Unknown	D7S518	13	2	0.15	Head&Neck	CR 55:1347
Unknown	D7S518	11	3	0.27	Prostate	CR 54:6370
Unknown	D7S515	13	3	0.23	Breast	PNAS 91:12155
Unknown	D7S496	17	8	0.47	Breast	PNAS 91:12155
Unknown	D7S496	10	4	0.31	Colon	CR 55:1347
Unknown	D7S496	10	1	0.1	Head&Neck	CR 55:1347
Unknown	D7S496	8	3	0.38	Prostate	CR 54:6370
22.3-31.2	D7S13	21	4	0.19	Breast	PNAS 91:12155
Unknown	D7S523	22	12	0.55	Breast	PNAS 91:12155
Unknown	D7S523	9	4	0.44	Colon	CR 55:1347
Unknown	D7S523	13	5	0.38	Head&Neck	CR 55:1347
Unknown	D7S523	7	2	0.29	Prostate	CR 54:6370
Unknown	D7S10	7	3	0.43	Breast	PNAS 91:12155
Unknown	D7S486	15	5	0.33	Breast	PNAS 91:12155
Unknown	D7S486	18	9	0.5	Colon	CR 55:1347
Unknown	D7S486	10	3	0.3	Head&Neck	CR 55:1347
Unknown	D7S486	6	2	0.33	Prostate	CR 54:6370
Unknown	D7S23	18	7	0.39	Breast	PNAS 91:12155
Unknown	D7S23	11	1	0.09	Ovary	BJC 69:429
Unknown	D7S23	15	2	0.13	Ovary	CR 53:2393
Unknown	D7S23	20	3	0.15	Uterus	CR 53:4294
31	MET	31	1	0.03	Breast	CR 53:4356
31	MET	121	49	0.4	Breast	N 339:140
31	MET	221	84	0.38	Breast	GCC 12:304
31	MET	18	8	0.44	Breast	PNAS 91:12155
31	MET	24	2	0.08	Breast	GCC 2:191
31	MET	15	0	0	Colon	CGG 48:167
31	MDR1-MET	12	0	0	Prostate	G 11:530
31	MET	9	3	0.33	Prostate	GCC 11:119

Chromosome 7 - q Arm

31	MET	14	1	0.07	Sarcoma	CR 52:2419
31	MET	37	7	0.2	Stomach	IJC 59:597
31	MET	1	0	0	Testis	CCG 52:72
31	MET	1	0	0	Testis	CCG 52:72
31	MET	1	0	0	Testis	CCG 52:72
Unknown	D7S633	7	4	0.57	Colon	CR 55:1347
Unknown	D7S633	6	2	0.33	Head&Neck	CR 55:1347
Unknown	D7S633	7	3	0.43	Prostate	CR 54:6370
Unknown	D7S677	9	6	0.67	Colon	CR 55:1347
Unknown	D7S677	10	4	0.4	Head&Neck	CR 55:1347
Unknown	D7S677	8	5	0.62	Prostate	CR 54:6370
Unknown	D7S655	8	4	0.5	Colon	CR 55:1347
Unknown	D7S655	7	3	0.43	Head&Neck	CR 55:1347
Unknown	D7S655	14	6	0.43	Prostate	CR 54:6370
Unknown	D7S522	11	9	0.82	Breast	PNAS 91:12155
Unknown	D7S522	10	8	0.8	Colon	CR 55:1347
Unknown	D7S522	15	8	0.53	Head&Neck	CR 55:1347
Unknown	D7S522	6	5	0.83	Prostate	CR 54:6370
Unknown	D7S480	21	9	0.43	Breast	PNAS 91:12155
Unknown	D7S480	27	4	0.15	Cervix	CR 56:197
Unknown	D7S480	16	7	0.44	Colon	CR 55:1347
Unknown	D7S480	10	4	0.4	Head&Neck	CR 55:1347
Unknown	D7S480	11	3	0.27	Prostate	CR 54:6370
Unknown	D7S487	15	4	0.27	Breast	PNAS 91:12155
Unknown	D7S487	8	2	0.25	Colon	CR 55:1347
Unknown	D7S487	10	0	0	Head&Neck	CR 55:1347
Unknown	D7S487	19	1	0.05	Leukemia	CR 55:5377
Unknown	D7S487	9	1	0.11	Prostate	CR 54:6370
31	CFTR	9	2	0.22	Ovary	BJC 69:429
Unknown	D7S490	14	5	0.36	Breast	PNAS 91:12155
Unknown	D7S490	10	4	0.4	Colon	CR 55:1347
Unknown	D7S490	12	4	0.33	Head&Neck	CR 55:1347
Unknown	D7S490	6	1	0.17	Prostate	CR 54:6370
31-32	D7S125	12	5	0.42	Breast	PNAS 91:12155
31-32	D7S125	15	2	0.13	Ovary	IJC 54:546
Unknown	D7S504	22	5	0.22	Breast	PNAS 91:12155
Unknown	D7S514	10	1	0.1	Breast	PNAS 91:12155
Unknown	D7S500	19	3	0.16	Breast	PNAS 91:12155
Unknown	D7S500	31	9	0.29	Cervix	CR 56:197
Unknown	D7S495	18	0	0	Breast	PNAS 91:12155
Unknown	D7S495	17	0	0	Head&Neck	CR 54:4756
Unknown	D7S495	20	1	0.05	Head&Neck	CR 54:4756
Unknown	D7S495	24	7	0.29	Head&Neck	CR 54:1152
Unknown	D7S498	26	1	0.03	Melanoma	CR 56:589
Unknown	D7S498	18	2	0.11	Breast	PNAS 91:12155
Unknown	D7S498	9	2	0.22	Colon	CR 55:1347

Chromosome 7 - q Arm

Unknown	D7S498	8	0	0	Head&Neck	CR 55:1347
Unknown	D7S498	4	0	0	Prostate	CR 54:6370
Unknown	D7S483	19	1	0.05	Breast	PNAS 91:12155
Unknown	D7S505	11	0	0	Breast	PNAS 91:12155
Unknown	D7S396	5	0	0	Brain	CR 49:6572
Unknown	D7S396	22	6	0.27	Breast	PNAS 91:12155
Unknown	D7S396	20	3	0.15	Breast	CR 50:7184
Unknown	D7S396	17	1	0.06	Esophageal	CR 54:2996
Unknown	D7S396	44	5	0.11	Esophageal	GCC 10:177
Unknown	D7S396	25	6	0.24	Kidney	CR 51:820
Unknown	D7S396	28	3	0.11	Liver	CR 51:89
Unknown	D7S396	34	5	0.15	Lung	CR 52:2478
Unknown	D7S396	19	4	0.21	Ovary	CR 51:5118
Unknown	D7S396	10	0	0	Sarcoma	CR 52:2419
36	D7S550	6	0	0	Colon	CR 55:1347
36	D7S550	28	3	0.11	Esophageal	IJC 69:1
36	D7S550	6	0	0	Head&Neck	CR 55:1347
36	D7S550	8	1	0.12	Prostate	CR 54:6370
36	D7S550	8	1	0.12	Prostate	CR 54:6370
Unknown	Unknown	31	0	0	Brain	CR 50:5784
Unknown	ABP1	6	2	0.33	Breast	PNAS 91:12155
32-qter	D7S228	18	2	0.11	Cervix	CR 54:4481
Unknown	D7S96	10	3	0.3	Cervix	GCC 9:119
3.3-ter	Unknown	32	0	0	Colon	BJC 59:750
Unknown	D7S368	21	0	0	Colon	CCG 48:167
Unknown	D7S22	11	0	0	Endocrine	N 328:524
Unknown	Unknown	10	0	0	Liver	BJC 64:1083
36	Unknown	12	0	0	Liver	BJC 61:1007
31.3-qter	Unknown	7	1	0.14	Pancreas	BJC 65:809
36	Unknown	1	0	0	Pancreas	CR 54:2761
31.3-qter	Unknown	19	2	0.11	Prostate	CSurveys 11:15
Unknown	Unknown	19	2	0.11	Prostate	PNAS 87:8751
3.3-ter	Unknown	9	0	0	Stomach	BJC 59:750
Unknown	D7S22	47	11	0.23	Stomach	IJC 59:597
Unknown	D7S22	41	10	0.24	Stomach	CR 51:2926
Unknown	D7S63	35	8	0.23	Stomach	IJC 59:597
Unknown	D7S64	16	0	0	Stomach	IJC 59:597
Unknown	D7S95	30	13	0.43	Stomach	IJC 59:597
Unknown	D7S22	22	2	0.09	Testis	GCC 13:249
32-qter	D7S228	23	2	0.09	Testis	O 9:2245
Unknown	TCBR	3	0	0	Testis	CCG 52:72
Unknown	TCBR	3	0	0	Testis	CCG 52:72
Unknown	TCBR	2	0	0	Testis	CCG 52:72
11.23	D7S440	19	1	0.05	Uterus	CR 54:4294
Unknown	D7S96	16	3	0.19	Uterus	GCC 9:119
SUM		2325	517	0.22		

Chromosome 7 - p Arm

Chromosome 8 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
21	D8S17	21	7	0.33	Breast	CR 53:4356
21	D8S17	3	1	0.33	Breast	CR 53:3804
21	D8S17	9	1	0.11	Ovary	IJC 54:546
Unknown	D8S264	30	6	0.2	Cervix	CR 56:197
Unknown	D8S262	5	2	0.4	Kidney	GCC 12:76
Unknown	D8S262	15	2	0.13	Leukemia	CR 55:5377
Unknown	D8S262	18	9	0.5	Prostate	CR 54:6061
23	D8S201	9	5	0.56	Colon	AJP 144:1
23	D8S201	28	6	0.21	Prostate	O 11:2121
23	D8S201	15	8	0.53	Prostate	AJP 144:1
23	D8S201	22	3	0.14	Prostate	CR 53:3869
23	D8S201	3	1	0.33	Sarcoma	AJP 144:1
23	D8S7	11	5	0.45	Colon	GCC 10:1
23	D8S7	18	6	0.33	Esophageal	CR 54:2996
23	D8S7	10	4	0.4	Ovary	CR 53:2393
23	D8S7	8	3	0.38	Prostate	GCC 3:215
23	D8S7	6	3	0.5	Prostate	O 11:530
23	D8S7	10	1	0.1	Sarcoma	CR 52:2419
Unknown	D8S277	18	0	0	Endocrine	CR 56:599
Unknown	D8S277	26	11	0.42	Prostate	CR 54:6061
23.1-.2	D8S337	18	5	0.28	Colon	CR 53:1172
23.1-.2	D8S337	15	7	0.47	Liver	GCC 7:152
23.1-.2	D8S337	3	0	0	Lung	GCC 8:75
23.1-.2	D8S337	14	6	0.43	Prostate	GCC 13:168
23.1-.2	D8S336	39	10	0.26	Colon	CR 53:1172
23.1-.2	D8S336	48	18	0.38	Liver	GCC 7:152
23.1-.2	D8S336	7	3	0.43	Lung	GCC 8:75
21.3-22	D8S335	53	18	0.34	Colon	CR 53:1172
21.3-22	D8S335	30	15	0.5	Colon	GCC 10:7
21.3-22	D8S335	46	17	0.37	Liver	GCC 7:152
21.3-22	D8S335	18	4	0.22	Liver	GCC 10:7
21.3-22	D8S335	27	12	0.44	Lung	GCC 10:7
21.3-22	D8S335	5	1	0.2	Lung	GCC 7:85
Unknown	D8S265	22	5	0.23	Cervix	CR 56:197
Unknown	D8S265	22	11	0.5	Prostate	CR 54:6061
22	CTSB	33	14	0.42	Colon	CR 53:1172
22	CTSB	23	7	0.3	Liver	GCC 7:152
11.21-.2	Unknown	33	10	0.3	Colon	CR 52:5368
11.21-.2	Unknown	34	8	0.24	Colon	CR 53:1172
11.21-.2	Unknown	34	0	0	Liver	GCC 7:152
11.21-.2	Unknown	12	0	0	Lung	GCC 7:85
Unknown	D8S254	13	4	0.31	Breast	CR 55:4995
Unknown	D8S261	16	1	0.06	Head&Neck	CR 54:4756
Unknown	D8S261	18	1	0.06	Head&Neck	CR 54:4756
Unknown	D8S261	20	8	0.4	Head&Neck	CR 53:1152
Unknown	D8S261	6	3	0.5	Kidney	GCC 12:76

Chromosome 8 - p Arm

Unknown	D8S261	24	3	0.12	Melanoma	CR 56:589
Unknown	D8S261	31	17	0.55	Prostate	CR 54:6061
22-pter	D8S163	44	19	0.43	Colon	CR 53:1172
22-pter	D8S163	31	14	0.45	Liver	GCC 7:152
22-pter	D8S163	14	3	0.21	Lung	GCC 8:75
22-pter	D8S163	1	0	0	Pancreas	CR 54:2761
22-pter	D8S163	23	14	0.61	Prostate	CR 53:3869
22-pTER	D8S163	18	9	0.5	Prostate	GCC 13:168
21.3-22	CI8-I344	71	25	0.35	Colon	GCC 10:7
21.3-22	CI8-I344	40	10	0.25	Liver	GCC 10:7
21.3-22	CI8-I344	30	8	0.27	Lung	GCC 10:7
21.3-22	CI8-2195	35	15	0.43	Colon	GCC 10:7
21.3-22	CI8-2195	32	7	0.22	Liver	GCC 10:7
21.3-22	CI8-2195	20	6	0.3	Lung	GCC 10:7
21.3-22	CI8-2014	24	7	0.29	Colon	GCC 10:7
21.3-22	CI8-2014	6	2	0.33	Liver	GCC 10:7
21.3-22	CI8-2014	17	7	0.41	Lung	GCC 10:7
21.3-22	CI8-2014	8	3	0.38	Prostate	GCC 13:168
21.3-22	D8S233	21	10	0.48	Colon	GCC 10:7
21.3-22	D8S233	24	11	0.46	Colon	CR 53:1172
21.3-22	D8S233	28	17	0.43	Liver	GCC 7:152
21.3-22	D8S233	14	5	0.36	Liver	GCC 10:7
21.3-22	D8S233	9	2	0.22	Lung	GCC 8:75
21.3-22	D8S233	7	3	0.43	Lung	GCC 10:7
Unknown	MSR	56	5	0.09	Breast	CR 52:5368
21.3-22	MSR	74	27	0.36	Colon	GCC 10:7
Unknown	MSR	26	12	0.46	Colon	CR 52:5368
22	MSR	74	28	0.38	Colon	CR 53:1172
Unknown	MSR	27	2	0.07	Kidney	CR 52:5368
Unknown	MSR	33	14	0.42	Liver	JJCR 84:893
22	MSR	87	37	0.43	Liver	GCC 7:152
21.3-22	MSR	54	10	0.19	Liver	GCC 10:7
Unknown	MSR	35	14	0.4	Lung	CR 52:5368
Unknown	MSR	21	9	0.43	Lung	GCC 8:75
21.3-22	MSR	38	16	0.42	Lung	GCC 10:7
Unknown	MSR	12	4	0.33	Ovary	CR 52:5368
21.3-22	MSR	29	18	0.62	Prostate	GCC 13:168
22	MSR	29	20	0.69	Prostate	CR 53:3869
Unknown	MSR	18	4	0.22	Stomach	CR 52:5368
21.3-22	Unknown	33	16	0.48	Colon	GCC 10:7
21.3-22	Unknown	9	3	0.33	Liver	GCC 10:7
21.3-22	Unknown	20	12	0.6	Lung	GCC 10:7
21.3-22	Unknown	18	11	0.61	Prostate	GCC 13:168
21.3-22	Unknown	21	9	0.43	Colon	GCC 10:7
21.3-22	Unknown	6	2	0.33	Liver	GCC 10:7
21.3-22	Unknown	22	15	0.68	Lung	GCC 10:7

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21.3-22	Unknown	42	19	0.45	Colon	GCC 10:7
21.3-22	Unknown	33	10	0.3	Liver	GCC 10:7
21.3-22	Unknown	21	10	0.48	Lung	GCC 10:7
21.3-22	Unknown	15	8	0.53	Prostate	GCC 13:168
21.3-22	Unknown	48	14	0.29	Colon	GCC 10:7
21.3-22	Unknown	39	9	0.23	Liver	GCC 10:7
21.3-22	Unknown	22	7	0.32	Lung	GCC 10:7
21.3-22	Unknown	15	8	0.53	Prostate	GCC 13:168
21.3-22	Unknown	49	22	0.45	Colon	GCC 10:7
21.3-22	Unknown	40	9	0.23	Liver	GCC 10:7
21.3-22	Unknown	23	7	0.3	Lung	GCC 10:7
21.3-22	Unknown	15	8	0.53	Prostate	GCC 13:168
21.3-22	Unknown	51	31	0.61	Colon	GCC 10:7
21.3-22	Unknown	54	16	0.3	Liver	GCC 10:7
21.3-22	Unknown	24	5	0.21	Lung	GCC 10:7
21.3-22	Unknown	20	8	0.4	Colon	GCC 10:7
21.3-22	Unknown	25	7	0.28	Liver	GCC 10:7
21.3-22	Unknown	17	4	0.24	Lung	GCC 10:7
21	Unknown	1	0	0	Pancreas	CR 54:2761
22	LPL	10	4	0.4	Colon	GCC 11:195
22	LPL	13	2	0.15	Colon	AJP 144:1
22	LPL	32	4	0.12	Colon	GCC 10:1
22	LPL	21	3	0.14	Colon	CR 53:1172
22	LPL	47	10	0.21	Colon	BJC 70:18
22	LPL	17	4	0.24	Leukemia	B 83:3449
22	LPL	38	19	0.5	Liver	GCC 7:152
22	LPL	6	4	0.67	Lung	CR 55:28
22	LPL	7	3	0.43	Lung	GCC 8:75
22	LPL	19	8	0.42	Prostate	AJP 144:1
22	LPL	13	5	0.38	Prostate	GCC 13:278
22	LPL	7	6	0.86	Prostate	GCC 3-215
22	LPL	32	15	0.47	Prostate	CR 53:3869
22	LPL	24	11	0.46	Prostate	O 11:2121
p22	LPL-GZ14-15	29	14	0.48	Prostate	CR 54:6061
22	LPL	2	0	0	Sarcoma	AJP 144:1
22	LPL	19	2	0.11	Uterus	CR 54:4294
Unknown	D8S258	16	3	0.19	Breast	CR 55:4995
Unknown	D8S282	27	13	0.48	Prostate	CR 54:6061
Unknown	D8S298	30	18	0.6	Prostate	CR 54:6061
21.3	D8S232	59	17	0.29	Colon	CR 53:1172
21.3	D8S232	40	13	0.33	Liver	GCC 7:152
21.3	D8S232	19	7	0.37	Lung	GCC 7:85
21.3	D8S334	47	16	0.34	Colon	CR 53:1172
21.3-22	D8S334	49	18	0.37	Colon	GCC 10:7
21.3-22	D8S334	37	8	0.22	Liver	GCC 10:7
21.3	D8S334	39	15	0.38	Liver	GCC 7:152

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21.3-22	D8S334	19	8	0.42	Lung	GCC 10:7
21.3	D8S334	6	2	0.33	Lung	GCC 7:85
21-3	D8S334	16	9	0.56	Prostate	GCC 13:168
21-23	EGR3	28	14	0.5	Colon	CR 53:1172
21-23	EGR3	33	12	0.36	Liver	GCC 7:152
21.2-.3	CI8-586	25	7	0.28	Colon	CR 53:1172
21.2-.3	CI8-586	20	9	0.45	Liver	GCC 7:152
21	D8S133	10	5	0.5	Prostate	GCC 11:119
21	D8S133	27	7	0.26	Prostate	O 11:2121
21	D8S133	29	16	0.55	Prostate	CR 54:6061
21.2-.3	D8S220	50	18	0.36	Colon	CR 53:1172
21.2-.3	D8S220	35	13	0.37	Colon	CR 52:5368
21.2-.3	D8S220	43	16	0.37	Liver	CR 52:5368
21.2-.3	D8S220	50	17	0.34	Liver	GCC 7:152
21.2-.3	D8S220	17	4	0.24	Lung	GCC 7:85
21.2-.3	D8S220	18	6	0.33	Prostate	GCC 13:168
21.2-.3	D8S220	27	16	0.59	Prostate	CR 53:3869
Unknown	SFTP2	40	11	0.28	Colon	GCC 10:1
Unknown	D8S136	20	7	0.35	Breast	CR 55:4995
Unknown	D8S136	11	6	0.55	Colon	GCC 11:195
Unknown	D8S136	1	1	1	Prostate	AJP 144:1
Unknown	D8S136	28	16	0.57	Prostate	CR 54:6061
21.1-.2	D8S221	53	14	0.26	Colon	CR 53:1172
21.1-.2	D8S221	41	10	0.24	Liver	GCC 7:152
21.1-.2	D8S221	10	0	0	Lung	GCC 7:85
21	NEFL	15	1	0.07	Brain	CR 50:5784
21	NEFL	2	1	0.5	Breast	CR 53:3804
21	NEFL	22	3	0.14	Cervix	CR 54:4481
21	NEFL	35	11	0.31	Colon	GCC 10:1
21	NEFL	8	4	0.5	Colon	GCC 11:195
21	NEFL	50	22	0.44	Colon	CR 53:1172
21	NEFL	47	19	0.4	Liver	GCC 7:152
21	NEFL	14	5	0.36	Lung	GCC 7:85
21	NEFL	6	2	0.33	Prostate	CR 53:3869
21	NEFL	8	7	0.88	Prostate	GCC 3:215
21	NEFL	19	8	0.42	Prostate	GCC 13:168
21	NEFL	21	9	0.43	Prostate	O 11:2121
21	NEFL	19	3	0.16	Testis	O 9:2245
Unknown	D8S137	16	10	0.62	Breast	CR 55:4995
Unknown	D8S137	85	29	0.34	Colon	BJC 70:18
Unknown	D8S137	1	1	1	Prostate	AJP 144:1
Unknown	D8S137	23	16	0.7	Prostate	CR 54:6061
Unknown	D8S137	2	2	1	Sarcoma	AJP 144:1
Unknown	D8S283	28	11	0.39	Prostate	CR 54:6061
p12	D8S87	14	2	0.14	Colon	AJP 144:1
p12	D8S87	24	9	0.38	Prostate	CR 54:6061

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p12	D8S87	20	5	0.25	Prostate	O 11:2121
p12	D8S87	18	4	0.22	Prostate	AJP 144:1
p12	D8S87	4	4	1	Sarcoma	AJP 144:1
p12	D8S87	25	5	0.2	Uterus	CR 54:4294
Unknown	D8S255	28	10	0.36	Prostate	CR 54:6061
Unknown	D8S255	10	1	0.1	Testis	LI 73:606
11.2	ANK1	78	18	0.23	Colon	BJC 70:18
11.2	ANK1	7	4	0.57	Prostate	AJP 144:1
11.2	ANK1	1	0	0	Sarcoma	AJP 144:1
11.21-.22	D8S194	40	6	0.15	Colon	CR 52:5368
11.21-.22	D8S194	40	5	0.12	Colon	CR 53:1172
11.21-.22	D8S194	45	5	0.11	Liver	CR 52:5368
11.21-.22	D8S194	45	5	0.11	Liver	GCC 7:152
11.21-.22	D8S194	26	3	0.12	Prostate	CR 53:3869
11.22-.23	D8S234	58	13	0.22	Colon	CR 53:1172
11.22-.23	D8S234	57	14	0.25	Liver	GCC 7:152
11.22-.23	D8S234	13	3	0.23	Lung	GCC 7:85
11.22-.23	D8S234	15	2	0.13	Prostate	GCC 13:168
23.2-.3	D8S140	33	6	0.18	Colon	CR 52:5368
23.2-.3	D8S140	29	8	0.28	Colon	CR 53:1172
23.2-.3	D8S140	39	7	0.18	Liver	GCC 7:152
23.2-.3	D8S140	39	7	0.18	Liver	CR 52:5368
23.2-.3	D8S140	38	4	0.11	Prostate	CR 53:3869
11.0-12	POLB	15	0	0	Colon	GCC 10:1
12-11.2	PLAT	7	2	0.29	Prostate	GCC 3:215
12-11.2	PLAT	18	0	0	Prostate	O 11:2121
11.23	D8S223	24	0	0	Colon	CR 53:1172
11.23	D8S223	37	0	0	Liver	GCC 7:152
11.23	D8S223	37	0	0	Liver	GCC 7:152
Unknown	D8S:262-261	26	17	0.65	Bladder	CR 55:5213
Unknown	D8S2	5	2	0.4	Breast	CR 53:3804
Unknown	D8S26	27	1	0.04	Breast	CR 53:4356
Unknown	D8S349	18	10	0.56	Breast	CR 55:4995
Unknown	D8S264-D8S265-D8S560	22	4	0.18	Kidney	PNAS 92:2854
Unknown	D8S264-D8S265-D8S560	6	1	0.17	Kidney	PNAS 92:2854
Unknown	D8S238	37	7	0.19	Liver	CR 52:5368
21	ARDRA5	19	5	0.26	Ovary	IJC 54:546
Unknown	D8S339	28	10	0.36	Prostate	CR 54:6061
22-21.3	D8S360	11	5	0.45	Prostate	O 11:2121
Unknown	D8S18	18	0	0	Testis	G 5:134
SUM		5603	1838	0.33		

Chromosome 8 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D8S260	28	7	0.25	Prostate	CR 54:6061
q22	D8S167	35	4	0.11	Prostate	CR 54:6061
Unknown	D8S257	16	0	0	Head&Neck	CR 54:4756
Unknown	D8S257	20	8	0.4	Head&Neck	CR 54:1152
Unknown	D8S257	14	0	0	Head&Neck	CR 54:4756
Unknown	D8S257	6	3	0.5	Kidney	GCC 12:76
Unknown	D8S257	26	2	0.08	Melanoma	CR 56:589
Unknown	D8S257	31	17	0.55	Prostate	CR 54:6061
Unknown	D8S273	30	6	0.2	Cervix	CR 56:1197
Unknown	D8S273	19	3	0.16	Head&Neck	CR 54:1152
Unknown	D8S284	21	5	0.24	Cervix	CR 56:1197
24	TG	2	0	0	Neuroblastoma	CR 49:1095
24	TG	14	4	0.29	Ovary	CR 53:2393
24	TG	9	0	0	Prostate	G 11:530
24	TG	8	0	0	Prostate	GCC 3:215
24	D8S39	14	1	0.07	Breast	CR 50:7184
24	D8S39	14	0	0	Cervix	CR 54:4487
24	D8S39	5	0	0	Cervix	GCC 9:119
24	D8S39	9	0	0	Esophageal	CR 51:2113
24	D8S39	22	0	0	Esophageal	CR 54:2996
24	D8S39	12	1	0.08	Kidney	CR 51:820
24	D8S39	20	4	0.2	Liver	CR 51:89
24	D8S39	1	1	1	Lung	CR 52:2478
24	D8S39	3	1	0.33	Lung	CR 52:2478
24	D8S39	8	1	0.12	Lung	CR 52:2478
24	D8S39	1	1	1	Lung	CR 52:2478
24	D8S39	16	5	0.31	Ovary	CR 51:5118
24	D8S39	7	0	0	Prostate	GCC 3:215
24	D8S39	17	2	0.12	Prostate	CR 53:3869
24	D8S39	14	1	0.07	Sarcoma	CR 52:2419
24	D8S39	18	4	0.22	Testis	O 9:2245
24	D8S39	8	0	0	Uterus	GCC 9:119
24	D8S39	8	0	0	Uterus	GCC 9:119
Unknown	Unknown	25	0	0	Brain	CR 50:5784
22-23	Unknown	12	0	0	Cervix	BJC 67:71
Unknown	D8S272	15	0	0	Endocrine	CR 56:599
Unknown	D8S177	42	4	0.1	Esophageal	GCC 10:177
Unknown	D8S272-D8S284	6	0	0	Kidney	PNAS 92:2854
Unknown	D8S272-D8S284	21	1	0.05	Kidney	PNAS 92:2854
Unknown	D8S:272-281	21	2	0.1	Leukemia	CR 55:5377
22-QTER	D8S161	19	5	0.26	Ovary	BJC 69:429
Unknown	D8S198	22	1	0.05	Uterus	CR 54:4294
Unknown	D8S84	20	0	0	Uterus	CR 54:4294
SUM		661	94	0.14		

Chromosome 9 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D9S143	33	17	0.52	Ovary	BJC 73:420
Unknown	D9S129	33	18	0.55	Ovary	BJC 73:420
22-24	D9S54	61	11	0.18	Bladder	CR 54:2848
22-PTER	D9S54	10	3	0.3	Ovary	BJC 69:429
Unknown	D9S132	5	1	0.2	Ovary	O 11:1249
Unknown	D9S132	3	0	0	Ovary	O 11:1249
Unknown	D9S199	21	15	0.71	Head&Neck	CR 54:1152
Unknown	D9S199	10	0	0	Ovary	O 11:1249
Unknown	D9S199	12	2	0.17	Ovary	O 11:1249
Unknown	D9S199	33	17	0.52	Ovary	BJC 73:420
Unknown	D9S324	23	2	0.09	Ovary	CR 55:2150
Unknown	D9S144	12	1	0.08	Ovary	O 11:1249
Unknown	D9S144	8	3	0.38	Ovary	O 11:1249
22	IFNA	40	26	0.65	Bladder	CR 54:2848
22	IFNA	12	1	0.08	Brain	CR 54:1397
22	IFNA	19	4	0.21	Brain	CR 54:1397
22	IFNA	89	21	0.24	Breast	IJC 64:378
Unknown	IFNA	13	4	0.31	Esophageal	CL 97:129
22	IFNA	2	0	0	Kidney	GCC 12:76
Unknown	IFNA	40	8	0.2	Kidney	JJCR 86:795
Unknown	IFNA	6	5	0.83	Lung	CR 55:28
Unknown	IFNA	15	8	0.53	Ovary	GO 55:245
Unknown	IFNA	28	3	0.11	Ovary	CR 55:2150
Unknown	IFNA	33	19	0.58	Ovary	BJC 73:420
22	IFNA	58	20	0.34	Ovary	AJHG 55:143
Unknown	IFNA	7	0	0	Ovary	O 11:1249
Unknown	IFNA	3	0	0	Ovary	O 11:1249
22	IFNA	19	5	0.26	Stomach	CR 55:1933
Unknown	IFNB	252	153	0.61	Bladder	CR 53:1230
22	IFNB1	252	153	0.61	Bladder	CR 53:1230
Unknown	IFNB	6	0	0	Breast	CR 53:4356
22	IFNB1	1	0	0	Breast	GCC 2:191
22	IFNB1	12	1	0.08	Cervix	CR 54:4481
22	IFNB1	42	5	0.12	Leukemia	AHEM 68:171
22	IFNB1	44	0	0	Leukemia	AHEM 68:171
22	IFNB1	6	0	0	Prostate	G 11:530
22	IFNB1	7	5	0.71	Testis	O 9:2245
Unknown	D9S156	126	30	0.24	Breast	IJC 64:378
Unknown	D9S156	11	4	0.36	Esophageal	CL 97:129
Unknown	D9S156	18	13	0.72	Head&Neck	CR 54:1152
Unknown	D9S156	3	0	0	Ovary	O 11:1249
Unknown	D9S156	13	4	0.31	Ovary	O 11:1249
21	D9S157	131	30	0.23	Breast	IJC 64:378
21	D9S157	30	5	0.17	Cervix	CR 56:197
21	D9S157	13	6	0.46	Esophageal	CL 97:129
21	D9S157	65	25	0.38	Esophageal	IJC 69:1

Chromosome 9 - p Arm

21	D9S157	5	1	0.2	Kidney	GCC 12:76
Unknown	D9S168	120	17	0.14	Breast	IJC 64:378
Unknown	D9S168	33	15	0.45	Ovary	BJC 73:420
21	CDKN2	109	20	0.18	Bladder	JNCI 87:1524
21	p15-p16	50	28	0.56	Esophageal	HMG 4:1883
21	CDKN2	55	1	0.02	Kidney	JJCR 86:795
21	CDKN2	34	7	0.21	Lung	GCC 14:164
21	CDKN2	50	24	0.48	Ovary	IJC 63:222
21	p15-p16	56	3	0.05	Sarcoma	CGC 86:136
21	MTS2	100	18	0.18	Bladder	JNCI 87:1524
21	D9S162	90	10	0.11	Breast	IJC 64:378
21	D9S162	9	3	0.33	Esophageal	CL 97:129
21	D9S162	33	4	0.12	Head&Neck	CR 54:4756
21	D9S162	41	13	0.32	Head&Neck	CR 54:4756
21	D9S162	4	0	0	Kidney	GCC 12:76
21	D9S162	33	17	0.52	Ovary	BJC 73:420
21	D9S162	12	1	0.08	Ovary	O 11:1249
21	D9S162	15	3	0.2	Ovary	O 11:1249
21	D9S171	139	26	0.2	Breast	IJC 64:378
21	D9S171	60	19	0.32	Esophageal	IJC 69:1
21	D9S171	11	4	0.36	Esophageal	CL 97:129
21	D9S171	3	0	0	Kidney	GCC 12:76
21	D9S171	12	3	0.25	Kidney	JJCR 86:795
Unknown	D9S:162-171	6	3	0.5	Kidney	GCC 12:76
21	D9S171	24	4	0.17	Lung	GCC 14:164
21	D9S171	8	5	0.62	Lung	CR 54:2307
Unknown	D9S:162-171	35	16	0.46	Melanoma	CR 56:589
21	D9S171	9	3	0.33	Ovary	O 11:1249
21	D9S171	33	16	0.48	Ovary	BJC 73:420
21	D9S171	15	1	0.07	Ovary	O 11:1249
Unknown	D9S126	252	152	0.6	Bladder	CR 53:1230
Unknown	D9S126	252	152	0.6	Bladder	CR 53:1230
Unknown	D9S126	80	15	0.19	Breast	IJC 64:378
Unknown	D9S126	16	3	0.19	Endocrine	CR 56:599
Unknown	IFNZa- D9S126	5	5	1	Lung	CR 55:513
Unknown	D9S126	9	0	0	Ovary	O 11:1249
Unknown	D9S126	11	1	0.09	Ovary	O 11:1249
Unknown	D9S126	51	17	0.33	Ovary	AJHG 55:143
Unknown	D9S126	30	3	0.1	Ovary	CR 55:2150
Unknown	D9S126	33	17	0.52	Ovary	BJC 73:420
Unknown	D9S126	33	18	0.55	Ovary	BJC 73:420
Unknown	D9S3	252	154	0.61	Bladder	CR 53:1230
21	D9S3	16	3	0.19	Bladder	CR 54:2848
21	D9S3	4	1	0.25	Breast	CR 53:3804
21	D9S169	22	4	0.18	Cervix	CR 56:197
21	D9S169	8	6	0.75	Lung	CR 54:2307

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21	S161	15	5	0.33	Esophageal	CL 97:129
21	S161	5	1	0.2	Kidney	GCC 12:76
21	S161	10	2	0.2	Ovary	O 11:1249
21	S161	14	0	0	Ovary	O 11:1249
Unknown	D9S104	117	20	0.17	Breast	IJC 64:378
Unknown	D9S104	63	27	0.43	Esophageal	IJC 69:1
Unknown	D9S104	33	15	0.45	Ovary	BJC 73:420
Unknown	D9S104	19	4	0.21	Uterus	CR 54:4294
21-qter	D9S52	12	5	0.42	Ovary	GO 55:245
Unknown	D9S165	4	0	0	Ovary	O 11:1249
Unknown	D9S165	8	0	0	Ovary	O 11:1249
Unknown	D9S200	11	2	0.18	Esophageal	CL 97:129
Unknown	D9S200	25	13	0.52	HeadGNeck	CR 54:1152
Unknown	D9S200	33	13	0.39	Ovary	BJC 73:420
Unknown	D9S200	13	1	0.08	Ovary	O 11:1249
Unknown	D9S200	13	4	0.31	Ovary	O 11:1249
12	D9S55	14	1	0.07	Brain	CR 54:1397
12	D9S55	18	2	0.11	Brain	CR 54:1397
12	D9S55	18	2	0.11	Brain	CR 54:1397
Unknown	D9S47	252	152	0.6	Bladder	CR 53:1230
Unknown	IFNa-D9S1751-736-1747-1748-1752-171	31	19	0.61	Bladder	CR 55:5213
Unknown	Unknown	12	0	0	Brain	CR 50:5784
Unknown	D9S18	30	17	0.57	Esophageal	GCC 10:177
Unknown	MTS1	5	5	1	Esophageal	O 9:3737
Unknown	D9S168-D9S166	5	2	0.4	Kidney	PNAS 92:2854
Unknown	D9S168-D9S166	19	3	0.16	Kidney	PNAS 92:2854
Unknown	D9S168-171	50	20	0.4	Leukemia	CR 55:5377
Unknown	Unknown	33	17	0.52	Lung	CR 54:2322
Unknown	D9S171-D9S126-D9S169	29	17	0.59	Lung	JCRCO 121:291
Unknown	D9S171-D9S126-D9S169	6	0	0	Lung	JCRCO 121:291
Unknown	D9S171-D9S126-D9S169	47	10	0.21	Lung	JCRCO 121:291
Unknown	OVC	15	5	0.33	Ovary	CR 53:2393
SUM		4921	1868	0.38		

Chromosome 9 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D9S15	70	37	0.53	Bladder	O 11:1671
Unknown	D9S15	11	1	0.09	Breast	CR 50:7184
13-21.1	D9S15	6	3	0.5	Cervix	GCC 9:119
13-21.1	D9S15	14	1	0.07	Esophageal	CR 54:2996
Unknown	D9S15	22	9	0.41	Esophageal	GCC 10:177
Unknown	D9S15	12	2	0.17	Kidney	CR 51:820
13-21.1	D9S15	6	1	0.17	Kidney	GCC 12:76
Unknown	D9S15	8	1	0.12	Lung	CR 52:2478
13-21.1	D9S15	14	5	0.36	Ovary	BJC 69:429
Unknown	D9S15	4	0	0	Ovary	CR 51:5118
Unknown	D9S15	16	2	0.12	Ovary	CR 55:2150
Unknown	D9S15	33	15	0.45	Ovary	BJC 73:420
Unknown	D9S15	10	3	0.3	Sarcoma	CR 52:2419
13-21.1	D9S15	10	2	0.2	Uterus	GCC 9:119
Unknown	D9S18	252	151	0.6	Bladder	CR 53:1230
Unknown	D9S18	7	0	0	Cervix	GCC 9:119
Unknown	D9S18	28	10	0.36	Esophageal	CR 54:2996
Unknown	D9S18	13	4	0.31	Ovary	IJC 54:546
Unknown	D9S18	16	1	0.06	Uterus	GCC 9:119
Unknown	D9S27	8	2	0.25	Testis	O 9:2245
Unknown	D9S103	70	36	0.51	Bladder	O 11:1671
Unknown	D9S103	33	16	0.48	Ovary	BJC 73:420
Unknown	D9S166	8	2	0.25	Ovary	O 11:1249
Unknown	D9S166	3	0	0	Ovary	O 11:1249
Unknown	ASSP3	252	155	0.62	Bladder	CR 53:1230
Unknown	ASSP3	8	0	0	Liver	CCG 48:72
11-22.0	ASSP3	19	7	0.37	Ovary	BJC 69:429
11-22.0	ASSP3	8	0	0	Stomach	CR 48:2988
Unknown	S153	70	37	0.53	Bladder	O 11:1671
pter-q11	D9S1	2	0	0	Cervix	CR 49:3598
pter-q11	D9S1	13	1	0.08	Colon	IJC 53:382
pter-q11	D9S1	7	0	0	Liver	JJCR 81:108
pter-q11	D9S1	5	0	0	Neuroblastoma	CR 49:1095
pter-q11	D9S1	1	0	0	Pancreas	CR 54:2761
pter-q11	D9S1	14	1	0.07	Stomach	CR 52:3099
pter-q11	D9S1	6	0	0	Uterus	CR 51:5632
Unknown	D9S167	70	38	0.54	Bladder	O 11:1671
Unknown	D9S201	70	36	0.51	Bladder	O 11:1671
Unknown	D9S201	26	7	0.27	Ovary	CR 55:2150
Unknown	D9S201	33	13	0.39	Ovary	BJC 73:420
Unknown	D9S283	70	37	0.53	Bladder	O 11:1671
Unknown	D9S283	33	13	0.39	Ovary	BJC 73:420
Unknown	D9S12	70	36	0.51	Bladder	O 11:1671
Unknown	D9S12	9	0	0	Colon	CCG 48:167
Unknown	D9S12	33	12	0.36	Ovary	BJC 73:420

Chromosome 9 - q Arm

Unknown	D9S12	13	6	0.46	Ovary	CR 55:2150
Unknown	D9S119	70	38	0.54	Bladder	O 11:1671
Unknown	D9S197	6	3	0.5	Kidney	GCC 12:76
Unknown	D9S197	26	5	0.19	Melanoma	CR 56:589
Unknown	D9S22	252	154	0.61	Bladder	CR 53:1230
Unknown	D9S176	70	38	0.54	Bladder	O 11:1671
Unknown	D9S176	6	1	0.17	Kidney	GCC 12:76
Unknown	D9S29	4	1	0.25	Head&Neck	CL 79:67
Unknown	D9S29	19	11	0.58	Ovary	CR 55:2150
Unknown	D9S109	70	37	0.53	Bladder	O 11:1671
Unknown	D9S109	5	1	0.2	Kidney	GCC 12:76
Unknown	D9S109	29	6	0.21	Ovary	CR 55:2150
Unknown	D9S127	70	36	0.51	Bladder	O 11:1671
Unknown	D9S127	24	7	0.29	Ovary	CR 55:2150
Unknown	D9S127	33	18	0.55	Ovary	BJC 73:420
Unknown	D9S53	70	38	0.54	Bladder	O 11:1671
Unknown	D9S53	19	3	0.16	Head&Neck	CR 54:1152
Unknown	D9S53	35	12	0.34	Ovary	CR 55:2150
Unknown	D9S53	33	19	0.58	Ovary	BJC 73:420
Unknown	D9S53	24	1	0.04	Uterus	CR 54:4294
Unknown	D9S58	70	37	0.53	Bladder	O 11:1671
Unknown	D9S58	27	7	0.26	Ovary	CR 55:2150
Unknown	D9S105	70	37	0.53	Bladder	O 11:1671
Unknown	HXB	70	39	0.56	Bladder	O 11:1671
Unknown	HXB	33	17	0.52	Ovary	BJC 73:420
Unknown	HXB	24	10	0.42	Ovary	CR 55:2150
Unknown	HXB	19	1	0.05	Uterus	CR 54:4294
Unknown	D9S155	33	15	0.45	Ovary	BJC 73:420
Unknown	D9S16	12	6	0.5	Ovary	CR 55:2150
Unknown	D9S59	70	37	0.53	Bladder	O 11:1671
Unknown	D9S59	33	18	0.55	Ovary	BJC 73:420
Unknown	D9S59	30	10	0.33	Ovary	CR 55:2150
Unknown	D9S154	70	38	0.54	Bladder	O 11:1671
Unknown	D9S154	34	5	0.15	Cervix	CR 56:197
Unknown	D9S302	36	4	0.11	Brain	CR 55:4696
Unknown	D9S302	36	4	0.11	Brain	CR 55:4696
Unknown	D9S258	70	35	0.5	Bladder	O 11:1671
33	GSN	70	39	0.56	Bladder	O 11:1671
33	GSN	17	3	0.18	Head&Neck	CR 54:1152
33	GSN	5	0	0	Kidney	GCC 12:76
33	GSN	18	8	0.44	Ovary	BJC 69:429
Unknown	GSN	33	16	0.48	Ovary	BJC 73:420
33	GSN	15	7	0.47	Ovary	CR 55:2150
Unknown	D9S99	252	154	0.61	Bladder	CR 53:1230
31-34	D9S28	39	5	0.13	Bladder	CR 54:2848
31-34	D9S28	1	1	1	Head&Neck	CL 79:67

Chromosome 9 - q Arm

Unknown	D9S60	70	36	0.51	Bladder	O 11:1671
Unknown	D9S61	70	38	0.54	Bladder	O 11:1671
34-QTER	D9S64	17	8	0.47	Ovary	BJC 69:429
Unknown	D9S64	18	10	0.56	Ovary	CR 55:2150
34.1	ABL	65	13	0.2	Bladder	CR 54:2848
34.1	ABL	70	37	0.53	Bladder	O 11:1671
34.1	ABL	33	15	0.45	Ovary	BJC 73:420
34.1	ABL	25	10	0.4	Ovary	CR 55:2150
34-qter	ASS	20	5	0.25	Bladder	CR 54:2848
34-qter	ASS	17	0	0	Brain	CR 54:1397
34-qter	ASS	12	0	0	Brain	CR 54:1397
34-qter	ASS	14	2	0.14	Lung	PN 84:9252
34-qter	ASS	34	13	0.38	Ovary	CR 55:2150
Unknown	D9S164	6	1	0.17	Kidney	PNAS 92:2851
Unknown	D9S164	20	3	0.15	Kidney	PNAS 92:2854
Unknown	D9S10	252	154	0.61	Bladder	CR 53:1230
34.3	D9S10	41	13	0.32	Bladder	CR 54:2848
34.3	D9S10	15	8	0.53	Ovary	CR 55:2150
Unknown	D9S66	70	38	0.54	Bladder	O 11:1671
Unknown	D9S14	252	151	0.6	Bladder	CR 53:1230
Unknown	D9S67	70	36	0.51	Bladder	O 11:1671
Unknown	D9S13	252	151	0.6	Bladder	CR 53:1230
34	D9S17	35	6	0.17	Breast	CR 50:7184
34	D9S17	21	16	0.76	Esophageal	GCC 10:177
34	D9S17	31	8	0.26	Lung	CR 52:2478
34	D9S17	20	2	0.1	Ovary	CR 51:5118
Unknown	D9S7	252	155	0.62	Bladder	CR 53:1230
34	D9S7	65	13	0.2	Bladder	CR 54:2848
34	D9S7	7	0	0	Brain	CR 49:6572
34	D9S7	21	2	0.1	Breast	GCC 2:191
Unknown	D9S7	44	6	0.14	Breast	CR 53:4356
34	D9S7	5	1	0.2	Breast	CR 53:3804
34	D9S7	3	2	0.67	Cervix	GCC 9:119
34	D9S7	33	5	0.15	Cervix	CR 54:4481
34	D9S7	20	1	0.05	Endocrine	GCC 13:9
Unknown	D9S7	9	0	0	Esophageal	CR 51:2113
34	D9S7	24	7	0.29	Esophageal	CR 54:2996
34	D9S7	10	1	0.1	Kidney	CR 51:820
34	D9S7	9	0	0	Liver	CR 51:89
34	D9S7	6	1	0.17	Liver	BJC 64:1083
34	D9S7	11	1	0.09	Liver	BJC 67:1007
Unknown	D9S7	32	6	0.19	Ovary	IJC 54:546
34	D9S7	6	1	0.17	Ovary	CR 55:2150
34	D9S7	2	0	0	Pancreas	CR 54:2761
34	D9S7	13	1	0.08	Pancreas	BJC 65:809
34	D9S7	12	0	0	Prostate	G 11:630

Chromosome 9 - q Arm

34	D9S7	13	2	0.15	Prostate	CSurveys 11:15
34	D9S7	11	2	0.18	Sarcoma	CR 52:2439
Unknown	D9S7	19	1	0.05	Testis	GCC 13:249
Unknown	D9S7	33	16	0.48	Testis	C 9,2245
34	D9S7	5	1	0.2	Uterus	GCC 9:119
Unknown	D9S11	252	153	0.61	Bladder	CR 53:1230
34	D9S7- D9S11-D9S13	252	149	0.59	Bladder	O 8:1083
34	D9S7- D9S11-D9S13	252	149	0.59	Bladder	O 8:1083
Unknown	GSN- D9S:15-12	28	17	0.61	Bladder	CR 55:5213
Unknown	Unknown	20	1	0.05	Brain	CR 50:5984
21.1-22.2	Unknown	14	1	0.07	Brain	CR 54:1397
21.1-22.2	Unknown	19	0	0	Brain	CR 54:1397
Unknown	D9S6	13	0	0	Colon	CCG 48:167
Unknown	D9S146	9	1	0.11	Endocrine	CR 56:399
Unknown	D9S160-180	44	26	0.59	Head&Neck	CR 54:4756
Unknown	D9S160-180	39	2	0.05	Head&Neck	CR 54:4756
Unknown	D9S:154-164-180	52	10	0.19	Leukemia	CR 55:5377
Unknown	Unknown	33	16	0.48	Lung	CR 54:2922
Unknown	D9S15-10	26	14	0.54	Ovary	CR 53:2393
Unknown	Unknown	19	2	0.11	Prostate	PNAS 87:8751
SUM		6593	3076	0.47		

Chromosome 10 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
pter-p11.2	D10S89	17	0	0	Uterus	CR 54:4794
Unknown	Unknown	38	15	0.39	Brain	CR 50:5784
Unknown	D10S109	7	0	0	Brain	CR 53:2386
Unknown	D10S109	6	2	0.33	Brain	CR 53:2386
11.2	D10S111	9	0	0	Brain	CR 53:2386
11.2	D10S111	6	0	0	Brain	CR 53:2386
pter-p11.2	D10S89	6	0	0	Brain	CR 53:2386
pter-p11.2	D10S89	16	1	0.06	Brain	CR 54:1397
pter-p11.2	D10S89	6	1	0.17	Brain	CR 53:2386
pter-p11.2	D10S89	13	0	0	Brain	CR 54:1397
Unknown	FNRB- D10S28	72	31	0.43	Brain	CR 56:164
pter-q13	D10 S28	32	4	0.12	Breast	CR 50:7184
Unknown	D10S15	15	0	0	Breast	GCC 2:191
pter-q13	D10 S28	42	9	0.21	Cervix	CR 54:4481
Unknown	D10S191	32	1	0.03	Cervix	CR 56:1972
13-12.2	D10S24	4	0	0	Cervix	CR 54:4481
Unknown	D10S28	7	1	0.14	Cervix	GCC 9:119
Unknown	D10S249	14	1	0.07	Endocrine	CR 56:599
pter-p11.2	D10S89	20	1	0.05	Endocrine	GCC 13:9
pTER-p13	D10S17	33	11	0.33	Esophageal	GCC 10:177
pTER-p13	D10S17	14	2	0.14	Esophageal	CR 54:2996
Unknown	D10S226	11	0	0	Head&Neck	CR 54:4756
Unknown	D10S226	12	0	0	Head&Neck	CR 54:4756
Unknown	D10S249	22	5	0.23	Head&Neck	CR 54:1152
pter-q13	D10 S28	31	3	0.1	Kidney	CR 51:5817
pter-q13	D10 S28	34	3	0.09	Kidney	CR 51:820
pTER-p13	D10S17	11	1	0.09	Kidney	CR 51:5817
Unknown	D10S226	6	3	0.5	Kidney	GCC 12:76
Unknown	D10S249-D10S191	21	0	0	Kidney	PNAS 92:285
Unknown	D10S249-D10S191	5	0	0	Kidney	PNAS 92:285
pter-q13	D10 S28	39	0	0	Liver	CR 51:99
pter-q13	D10 S28	35	5	0.14	Lung	CR 52:2478
11-23.0	D10S14	8	4	0.5	Melanoma	GCC 8:178
Unknown	D10S15	5	3	0.6	Melanoma	GCC 8:178
Unknown	D10S226	23	4	0.17	Melanoma	CR 56:589
Unknown	D10S28	14	5	0.36	Melanoma	GCC 8:178
Unknown	D10S33	3	0	0	Melanoma	GCC 8:178
pter-p11.2	D10S89	10	4	0.4	Melanoma	GCC 8:178
pter-q13	D10 S28	27	3	0.11	Ovary	CR 51:5118
pter-q13	D10 S28	35	5	0.14	Ovary	IJC 54:546
Unknown	D10S13-28	33	4	0.12	Ovary	CR 53:2393
pter-q13	D10 S28	7	3	0.43	Pancreas	CR 54:2761
pter-q13	D10 S28	19	4	0.21	Prostate	BJU 73:390
11-23.0	D10S14	11	3	0.27	Prostate	GCC 3:215
13-pter	D10S17	18	0	0	Prostate	CSurveys 11

Chromosome 10 - p Arm

pTER-p13	D10S17	11	6	0.55	Prostate	G 11:530
pter-p12	D10S17	13	5	0.38	Prostate	GCC 7:215
pTER-p13	D10S17	18	0	0	Prostate	PNAS 87:875
13-12.2	D10S21	11	4	0.29	Prostate	GCC 5:215
pter-p12	D10S17	14	5	0.36	Sarcoma	CR 52:2419
pter-q13	D10 S28	47	5	0.11	Testis	O 9:2345 7.
Unknown	D10S28	14	4	0.29	Uterus	GCC 9:119
pter-p11.2	D10S89	17	0	0	Uterus	CR 50:294
SUM		980	172	0.18		

Chromosome 10 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
24-TER	PLAU	5	0	0	Uterus	CR 51:5632
Unknown	Unknown	37	14	0.38	Brain	CR 50:5784
12-qter	Unknown	12	0	0	Brain	CR 54:1397
11.2	Unknown	12	0	0	Brain	CR 54:1397
11.2	Unknown	17	2	0.12	Brain	CR 54:1397
12-qter	Unknown	15	1	0.07	Brain	CR 54:1397
Unknown	D10S.25-22-1	64	21	0.33	Brain	CR 56:164
22-23	D10S1	5	0	0	Brain	CR 48:5546
22-23	D10S1	4	0	0	Brain	CR 48:5546
22-23	D10S1	10	10	1	Brain	CR 48:5546
Unknown	D10S169	7	0	0	Brain	CR 53:2386
Unknown	D10S169	5	2	0.4	Brain	CR 53:2386
22-23	D10S4	21	20	0.95	Brain	CR 48:5546
22-23	D10S4	6	0	0	Brain	CR 48:5546
22-23	D10S4	11	0	0	Brain	CR 48:5546
24-TER	PLAU	10	0	0	Brain	CR 48:5546
24-TER	PLAU	5	0	0	Brain	CR 48:5546
24-TER	PLAU	14	14	1	Brain	CR 48:5546
22-23	D10S1	18	2	0.11	Breast	CR 53:4356
26	D10S25	6	2	0.33	Breast	CR 53:3804
26	D10S25	23	2	0.09	Breast	CR 50:7184
26	D10S25	30	5	0.17	Breast	GCC 2:191
22-23	D10S4	18	4	0.22	Breast	GCC 2:191
Unknown	D10S205	32	4	0.12	Cervix	CR 56:197
26	D10S25	32	9	0.28	Cervix	CR 54:4481
26	D10S25	8	2	0.25	Cervix	GCC 9:119
11	D10S30	8	2	0.25	Cervix	GCC 9:119
21.1	D10S5	17	1	0.06	Cervix	CR 54:4481
24-TER	PLAU	4	1	0.25	Cervix	CR 48:3590
24-TER	PLAU	6	0	0	Colon	IJC 53:382
Unknown	D10S187	22	2	0.09	Endocrine	CR 56:599
26	D10S25	25	4	0.16	Esophageal	CR 54:2996
26	D10S25	36	6	0.17	Esophageal	GCC 10:177
26	D10S25	17	0	0	Esophageal	CR 51:2113
Unknown	D10S185	12	3	0.25	Head&Neck	CR 54:4756
Unknown	D10S185	21	0	0	Head&Neck	CR 54:4756
Unknown	D10S221	24	5	0.21	Head&Neck	CR 54:1152
22-25	D10S13	32	9	0.28	Kidney	CR 51:5817
21	D10S14	17	5	0.29	Kidney	CR 51:5817
Unknown	D10S185	6	3	0.5	Kidney	GCC 12:76
21-TER	D10S20	25	8	0.32	Kidney	CR 51:5817
Unknown	D10S212-D10S190	19	1	0.05	Kidney	PNAS 92:2854
Unknown	D10S212-D10S190	5	0	0	Kidney	PNAS 92:2854
21	D10S22	10	3	0.3	Kidney	CR 51:5817
21	D10S23	15	3	0.2	Kidney	CR 51:5817
26	D10S25	30	10	0.33	Kidney	CR 51:5817

Chromosome 10 - q Arm

26	D10S25	21	6	0.29	Kidney	CR 51:820
22-25	D10S27	26	3	0.12	Kidney	CR 51:5817
11	D10S30	13	2	0.15	Kidney	CR 51:5817
26	D10S36	27	5	0.19	Kidney	CR 51:5817
Unknown	D10S201	19	1	0.05	Leukemia	CR 55:5377
Unknown	Unknown	16	0	0	Liver	CR 51:89
22-23	D10S1	3	1	0.33	Liver	CCG 48:72
26	D10S25	24	6	0.25	Liver	CR 51:89
Unknown	D10S26	24	6	0.25	Liver	CR 51:89
24-TER	PLAU	20	0	0	Liver	JJCR 81:108
26	D10S25	25	15	0.2	Lung	CR 52:2478
Unknown	ATC	9	4	0.44	Melanoma	CR 54:3111
Unknown	CHLC.GCAA2F11	14	6	0.43	Melanoma	CR 54:3111
Unknown	D10S108	5	1	0.2	Melanoma	CR 54:3111
Unknown	D10S110	4	2	0.5	Melanoma	CR 54:3111
Unknown	D10S168	8	5	0.62	Melanoma	CR 54:3111
Unknown	D10S169	8	1	0.12	Melanoma	CR 54:3111
Unknown	D10S185	29	9	0.31	Melanoma	CR 56:589
Unknown	D10S187	12	3	0.25	Melanoma	CR 54:3111
21-22	D10S19	8	3	0.38	Melanoma	GCC 8:178
21-TER	D10S20	4	3	0.75	Melanoma	GCC 8:178
Unknown	D10S221	12	4	0.33	Melanoma	CR 54:3111
26	D10S36	9	4	0.44	Melanoma	GCC 8:178
Unknown	D10S610	9	4	0.44	Melanoma	CR 54:3111
Unknown	D10S86	6	3	0.5	Melanoma	CR 54:3111
24-TER	PLAU	5	0	0	Neuroblastoma	CR 49:1095
Unknown	D10S1-20	19	2	0.11	Ovary	CR 53:2393
Unknown	D10S173	16	3	0.19	Ovary	BJC 69:429
26	D10S25	34	4	0.12	Ovary	IJC 54:546
26	D10S25	24	5	0.21	Ovary	CR 51:5118
26	D10S25	4	0	0	Pancreas	CR 54:2761
Unknown	Unknown	24	7	0.29	Prostate	CSurveys 11:15
22-23	D10S1	2	0	0	Prostate	GCC 3:215
21-22	D10S19	8	1	0.12	Prostate	GCC 3:215
21-22	D10S19	7	0	0	Prostate	GCC 11:119
21-TER	D10S20	8	2	0.25	Prostate	GCC 3:215
26	D10S25	8	3	0.38	Prostate	GCC 11:119
26	D10S25	13	4	0.31	Prostate	G 11:530
26	D10S25	13	4	0.31	Prostate	GCC 3:215
Unknown	D10S26	9	2	0.22	Prostate	GCC 3:215
22-23	D10S4	10	1	0.1	Prostate	GCC 3:215
26	D10S90	19	8	0.42	Prostate	BJU 73:390
26	OAT	25	7	0.28	Prostate	PNAS 87:8751
24-TER	PLAU	9	2	0.22	Prostate	GCC 3:215
26	D10S25	17	9	0.53	Sarcoma	CR 52:2419
Unknown	Unknown	2	0	0	Stomach	CR 48:2988

Chromosome 10 - q Arm

Unknown	D10S26	20	0	0	Stomach	CR 51:2926
26	D10S25	34	9	0.26	Testis	O 9:2245
11.2	PTC	1	0	0	Testis	CCG 52:72
11.2	PTC	2	1	0.5	Testis	CCG 52:72
11.2	PTC	1	0	0	Testis	CCG 52:72
Unknown	D10S173	16	1	0.06	Uterus	CR 54:4294
26	D10S25	14	6	0.43	Uterus	GCC 9:119
11	D10S30	12	3	0.25	Uterus	GCC 9:119
24_TER	PLAU	5	0	0	Uterus	CR 51:5632
SUM		1509	351	0.23		

Chromosome 11 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	HRAS1-D11S2	17	7	0.41	Bladder	CR 51:5485
15.5	HRAS	7	2	0.29	Brain	CR 49:6572
15.5	HRAS	30	3	0.1	Breast	GCC 1:113
15.5	HRAS	24	3	0.12	Breast	CR 53:4486
15.5	HRAS	5	0	0	Breast	GCC 2:191
15.5	HRAS	68	21	0.31	Breast	GCC 12:304
15.5	HRAS	30	8	0.27	Breast	IJC 53:19
15.5	HRAS	29	5	0.17	Breast	JJCR 84:11
15.5	HRAS	7	1	0.14	Breast	CR 50:3804
15.5	HRAS	33	1	0.03	Breast	CR 53:4356
15.5	HRAS	37	9	0.24	Breast	GC 5:553
15.5	HRAS	6	0	0	Cervix	CR 49:3598
15.5	HRAS	18	6	0.33	Cervix	PNAS 87:469
15.5	HRAS	15	1	0.07	Cervix	BJC 67:71
15.5	HRAS	10	0	0	Colon	N 331:213
15.5	HRAS	16	0	0	Colon	CCG 48:167
15.5	HRAS	9	0	0	Colon	N 331:213
15.5	HRAS	9	1	0.11	Esophageal	CR 51:2113
15.5	HRAS	21	4	0.19	Esophageal	GCC 10:177
15.5	HRAS	20	8	0.4	Esophageal	CR 54:2996
15.5	HRAS	12	1	0.09	Head&Neck	CR 52:3191
15.5	HRAS	3	0	0	Kidney	CMB 38:59
15.5	HRAS	14	1	0.07	Kidney	CR 51:1071
15.5	HRAS	5	0	0	Kidney	CMB 38:59
15.5	HRAS	13	4	0.31	Leukemia	P 75:819
15.5	HRAS	5	0	0	Liver	JJCR 81:10
15.5	HRAS	3	0	0	Liver	BJC 67:100
15.5	HRAS	13	0	0	Liver	GCC 1:312
15.5	HRAS	4	0	0	Liver	PNAS 86:88
15.5	HRAS	10	5	0.5	Liver	CCG 48:72
15.5	HRAS	5	0	0	Liver	BJC 64:108
15.5	HRAS	47	7	0.15	Lung	GCC 10:183
15.5	HRAS	39	7	0.18	Lung	CR 54:1145
15.5	HRAS	13	5	0.38	Lung	PN 86:5099
15.5	HRAS	13	6	0.46	Lung	PN 91:5513
15.5	HRAS	2	1	0.5	Lung	PN 91:5513
15.5	HRAS	12	6	0.5	Lung	PN 86:5099
15.5	HRAS	7	1	0.14	Lung	NEJ 317:11
15.5	HRAS	5	2	0.4	Lung	PN 86:5099
15.5	HRAS	13	3	0.23	Lung	PN 84:9252
15.5	HRAS	6	2	0.33	Lung	PN 91:5513
15.5	HRAS	4	0	0	Neuroblastoma	CR 49:1095
15.5	HRAS	25	10	0.4	Ovary	GO 47:139
15.5	HRAS	15	4	0.27	Ovary	GO 55:245
15.5	HRAS	11	5	0.45	Ovary	CR 50:2721

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15.5	HRAS	11	2	0.18	Ovary	IJC 54:546
15.5	HRAS	27	12	0.44	Ovary	C 72:2423
15.5	HRAS	10	5	0.5	Ovary	CR 49:1220
15.5	HRAS	13	2	0.15	Ovary	BJC 67:268
15.5	HRAS	19	9	0.47	Ovary	BRJ 66:103
15.5	HRAS	5	2	0.4	Pancreas	BJC 65:809
15.5	HRAS	20	7	0.35	Pediatric	CR 50:3279
15.5	HRAS	15	5	0.33	Pediatric	BG 97:163
15.5	HRAS	9	0	0	Prostate	GCC 11:119
15.5	HRAS	11	5	0.45	Sarcoma	CR 52:2419
15.5	HRAS	11	5	0.45	Sarcoma	CR 52:2419
15.5	HRAS	9	0	0	Stomach	CR 48:2988
15.5	HRAS	28	1	0.04	Stomach	CR 51:2926
15.5	HRAS	19	7	0.37	Stomach	HG 92:244
15.5	HRAS	6	0	0	Stomach	HG 89:445
15.5	HRAS	15	7	0.47	Testis	GCC 9:153
15.5	HRAS	5	2	0.4	Testis	CCG 52:72
15.5	HRAS	12	3	0.25	Testis	GCC 9:153
15.5	HRAS	13	5	0.38	Testis	G 5:134
15.5	HRAS	17	3	0.18	Testis	JU 153:168
15.5	HRAS	15	0	0	Testis	GCC 13:249
15.5	HRAS	15	5	0.33	Testis	GCC 2:85
15.5	HRAS	3	1	0.33	Testis	CCG 52:72
15.5	HRAS	3	1	0.33	Testis	CCG 52:72
15.5	HRAS	9	1	0.11	Uterus	CR 51:5632
15.5	IGF2	7	2	0.29	Bladder	HG 91:455
15.5	IGF2	15	1	0.07	Breast	GE 5:554
15.5	IGF2	13	3	0.23	Cervix	O 12:423
15.5	IGF2	1	0	0	Lung	PN 91:5513
15.5	IGF2	7	0	0	Lung	PN 91:5513
15.5	IGF2	1	0	0	Lung	PN 91:5513
15.5	IGF2	14	6	0.43	Ovary	BRJ 66:103
15.5	IGF2	9	6	0.67	Testis	JU 153:168
15.5	MUC2	17	2	0.12	Testis	GCC 13:249
15.5	H19	14	2	0.14	Cervix	O 12:423
Unknown	D11S922	46	8	0.17	Head&Neck	CR 54:4756
Unknown	D11S922	40	1	0.03	Head&Neck	CR 54:4756
Unknown	D11S922	6	1	0.17	Kidney	PNAS 92:28
Unknown	D11S922	19	1	0.05	Kidney	PNAS 92:28
Unknown	D11S922	8	4	0.5	Pediatric	HG 97:163
Unknown	D11S922	49	16	0.33	Stomach	CR 56:268
Unknown	D11S1318	16	7	0.44	Pediatric	HG 97:163
Unknown	D11S1318	15	9	0.6	Stomach	CR 56:268
15.5	INS	33	3	0.1	Breast	CR 50:7184
15.5	INS	23	4	0.17	Breast	GCC 2:191
15.5	INS	33	3	0.1	Breast	CR 50:7184

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15.5	INS	3	0	0	Cervix	CR 49:3598
15.5	INS	3	0	0	Cervix	CR 49:3598
15.5	INS	15	3	0.2	Colon	IJC 53:382
15.5	INS	15	3	0.2	Colon	IJC 53:382
15.5	INS	8	2	0.25	Endocrine	CR 51:1154
15.5	INS	22	5	0.23	Kidney	CR 51:620
15.5	INS	7	0	0	Kidney	CMB 38:59
15.5	INS	21	3	0.14	Kidney	CR 51:1071
15.5	INS	7	0	0	Kidney	CMB 38:59
15.5	INS	22	5	0.23	Kidney	CR 51:620
15.5	INS	6	0	0	Liver	GCC 1:312
15.5	INS	6	1	0.17	Liver	CR 51:4961
15.5	INS	9	0	0	Liver	JJCR 81:10
15.5	INS	11	3	0.27	Liver	CR 51:397
15.5	INS	10	2	0.2	Liver	CCG 48:72
15.5	INS	10	3	0.3	Lung	PN 86:5099
15.5	INS	5	1	0.2	Lung	PN 86:5099
15.5	INS	14	7	0.5	Lung	PN 86:5099
15.5	INS	33	12	0.36	Lung	GCC 10:183
15.5	INS	8	1	0.12	Lung	PN 91:5513
15.5	INS	2	0	0	Lung	PN 91:5513
15.5	INS	8	1	0.12	Lung	PN 91:5513
15.5	INS	12	3	0.25	Lung	PN 84:9252
15.5	INS	6	0	0	Neuroblastoma	CR 49:1095
15.5	INS	5	0	0	Ovary	CR 50:2724
15.5	INS	13	7	0.54	Ovary	GO 55:235
15.5	INS	32	12	0.38	Ovary	C 72:2423
15.5	INS	27	7	0.26	Ovary	CR 51:5110
15.5	INS	20	7	0.35	Ovary	BRJ 66:103
15.5	INS	23	10	0.43	Pediatric	CR 50:3299
15.5	INS	9	0	0	Stomach	CR 48:2988
15.5	INS	2	0	0	Stomach	CR 52:5099
15.5	INS	15	4	0.27	Testis	GCC 7:96
15.5	INS	5	1	0.2	Testis	CCG 52:72
15.5	INS	2	0	0	Testis	CCG 52:72
15.5	INS	5	2	0.4	Testis	CCG 52:72
15.5	INS	15	3	0.2	Testis	G 5:134
15.5	INS	18	3	0.17	Testis	GCC 13:249
15.5	INS	3	0	0	Uterus	CR 51:5632
15.5	TH	15	1	0.07	Brain	CR 54:1397
15.5	TH	21	3	0.14	Brain	CR 54:1397
15.5	TH	16	4	0.25	Breast	BMC 4:1889
15.5	TH	14	4	0.29	Breast	CR 54:6270
15.5	TH	41	11	0.27	Breast	CR 53:4486
15.5	TH	14	1	0.07	Cervix	BJC 67:71
15.5	TH	20	8	0.4	Cervix	PNAS 91:169

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15.5	TH	10	0	0	Kidney	CMB 38:59
15.5	TH	8	0	0	Kidney	CMB 38:59
15.5	TH	8	1	0.12	Lung	PN 91:5513
15.5	TH	10	0	0	Lung	PN 91:5513
15.5	TH	2	0	0	Lung	PN 91:5513
15.5	TH	20	7	0.35	Ovary	GO 57:2071
15.5	TH	23	9	0.39	Pediatric	HG 97:163
15.5	DRD4	7	1	0.14	Lung	PN 91:5513
15.5	DRD4	3	0	0	Lung	PN 91:5513
Unknown	D11S454	13	6	0.46	Liver	CR 53:59
Unknown	D11S454	18	4	0.22	Lung	CR 52:2478
Unknown	D11S454	11	0	0	Ovary	CR 51:5598
15.5	D11S988	1	0	0	Lung	PN 91:5513
15.5	D11S988	2	0	0	Lung	PN 91:5513
15.5	D11S988	17	6	0.35	Pediatric	HG 97:163
15.5	D11S988	17	12	0.71	Stomach	CR 56:2083
15.5	D11S12	32	5	0.16	Breast	GE 5:554
15.5	D11S12	3	1	0.33	Breast	GCC 2:291
15.5	D11S12	0	0	0	Cervix	CR 49:3598
15.5	D11S12	12	5	0.42	Cervix	CR 54:6481
15.5	D11S12	33	6	0.18	Esophageal	CR 54:2996
15.5	D11S12	15	3	0.2	Kidney	CR 51:1971
15.5	D11S12	11	8	0.73	Lung	PN 91:5513
15.5	D11S12	1	1	1	Lung	PN 91:5513
15.5	D11S12	4	2	0.5	Lung	PN 91:5513
15.5	D11S12	4	2	0.5	Ovary	BRJ 66:103
15.5	D11S12	3	1	0.33	Stomach	HG 89:445
15.5	D11S12	1	1	1	Testis	CCG 52:72
15.5	D11S12	20	6	0.3	Testis	O 9:2245
15.5	D11S12	1	0	0	Testis	CCG 52:72
15.5	D11S12	8	3	0.38	Testis	JU 153:168
15.5	D11S12	5	1	0.2	Uterus	CR 51:5639
15.5-15.4	RRM1	42	7	0.17	Lung	GCC 10:183
15.5	HBB	27	9	0.33	Breast	CR 53:4486
15	HBG	6	0	0	Liver	PNAS 86:88
15.5	HBB	2	0	0	Lung	PN 91:5513
15.5	HBB	4	0	0	Lung	PN 91:5513
15.5	HBB	6	0	0	Lung	PN 91:5513
15.5	HBG2	2	0	0	Lung	PN 86:5099
15.5	HBG2	8	4	0.5	Lung	PN 86:5099
15.5	HBG2	5	4	0.8	Lung	PN 86:5099
15.5	HBB	21	7	0.33	Pediatric	HG 97:163
15	GLOBIN	30	4	0.13	Breast	GE 5:554
15	GLOBIN	16	4	0.25	Ovary	BRJ 66:103
Unknown	GLOBIN	14	5	0.36	Ovary	BRJ 66:103
Unknown	GLOBIN	13	2	0.15	Ovary	BRJ 66:103

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15.5	D11S932	5	0	0	Lung	PN 91:5513
15.5	D11S932	9	1	0.11	Lung	PN 91:5513
15.5	D11S932	1	0	0	Lung	PN 91:5513
Unknown	D11S969	27	13	0.48	Stomach	CR 56:268
Unknown	D11S569	24	3	0.12	Uterus	CR 54:4294
pter-15.4	PTH	11	1	0.09	Bladder	HG 91:455
pter-15.4	PTH	15	1	0.07	Kidney	CR 51:1071
pter-15.4	PTH	7	0	0	Liver	GCC 1:312
pter-15.4	PTH	8	1	0.12	Liver	CCG 48:72
pter-15.4	PTH	7	1	0.14	Lung	PN 91:5513
pter-15.4	PTH	5	1	0.2	Lung	PN 91:5513
pter-15.4	PTH	29	9	0.31	Ovary	G 72:2423
pter-15.4	PTH	7	0	0	Testis	GCC 7:96
pter-15.4	PTH	3	2	0.67	Testis	CCG 52:72
pter-15.4	PTH	1	0	0	Testis	CCG 52:72
pter-15.4	PTH	1	0	0	Testis	CCG 52:72
pter-15.4	PTH	15	6	0.4	Testis	JU 153:168
13-15.1	D11S419	14	6	0.43	Ovary	BJC 69:429
Unknown	D11S902	28	8	0.29	Cervix	PNAS 91:69
14-qter	D11S899	23	4	0.17	Head&Neck	CR 54:1152
14-qter	D11S899	6	0	0	Kidney	GCC 12:76
15.5	D11S861	21	5	0.24	Endocrine	CR 56:599
15.5	D11S861	1	0	0	Lung	PN 91:5513
15.5	D11S861	9	0	0	Lung	PN 91:5513
15.5	D11S861	7	0	0	Lung	PN 91:5513
Unknown	D11S860	27	9	0.33	Breast	CR 53:4486
15.5	D11S860	36	10	0.28	Breast	Unknown
15.5	D11S860	36	10	0.28	Breast	CR 54:6270
15.5	D11S860	7	0	0	Lung	PN 91:5513
15.5	D11S860	7	0	0	Lung	PN 91:5513
15.5	D11S860	2	0	0	Lung	PN 91:5513
15.5	D11S860	5	0	0	Lung	PN 91:5513
15.5	D11S860	5	0	0	Lung	PN 91:5513
15.5	D11S860	2	0	0	Lung	PN 91:5513
15.5	D11S860	16	6	0.38	Pediatric	HG 97:163
15.5	D11S860	44	16	0.36	Stomach	CR 56:268
15.4	CALCA	6	0	0	Bladder	HG 91:455
15.4	CALCA	17	11	0.65	Breast	GCC 2:191
15.4	CALCA	22	0	0	Breast	GE 5:554
15.4	CALCA	10	3	0.3	Cervix	BJC 67:71
15.4	CALCA	5	0	0	Kidney	CMB 38:59
15.4	CALCA	4	0	0	Kidney	CMB 38:59
15.4	CALCA	7	0	0	Liver	CCG 48:72
15.4	CALCA	10	1	0.1	Liver	CR 51:4367
15.4	CALCA	3	0	0	Liver	GCC 1:312
15.4	CALCA	6	0	0	Lung	PN 86:5099

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15.4	CALCA	6	1	0.17	Lung	PN 91:5513
15.4	CALCA	6	2	0.33	Lung	PN 86:5099
15.4	CALCA	2	0	0	Lung	PN 86:5099
15.4	CALCA	3	1	0.33	Lung	PN 91:5513
15.4	CALCA	10	3	0.3	Ovary	C 72:2423
15.4	CALCA	15	6	0.4	Ovary	BBRC 66:103
15.4	CALCA	7	0	0	Stomach	HG 89:445
15.4	CALCA	6	3	0.5	Testis	GCC 7:96
Unknown	D11S929	33	3	0.09	Cervix	CR 56:197
Unknown	D11S929	17	4	0.24	Pediatric	HG 97:163
13	D11S323	3	1	0.33	Lung	PN 91:5513
13	D11S323	3	1	0.33	Lung	PN 91:5513
13	D11S907	16	3	0.19	Endocrine	CR 56:599
13	D11S907	14	1	0.07	Head&Neck	CR 54:1152
13	D11S907	1	0	0	Kidney	GCC 12:76
13	D11S16	17	4	0.24	Cervix	PNAS 91:169
13	D11S16	30	4	0.13	Colon	IJC 53:382
13	D11S16	5	0	0	Kidney	CMB 38:59
13	D11S16	4	0	0	Kidney	CMB 38:59
13	D11S16	6	0	0	Liver	GCC 1:317
13	D11S16	7	2	0.29	Lung	PN 91:5513
13	D11S16	1	1	1	Lung	PN 91:5513
13	D11S16	10	7	0.7	Lung	PN 91:5513
13	D11S16	25	2	0.08	Ovary	IJC 54:546
13	D11S16	23	6	0.26	Ovary	BRJ 66:103
13	D11S16	7	4	0.57	Testis	JU 153:168
13	D11S16	12	3	0.25	Testis	GCC 9:153
13	D11S16	12	5	0.42	Testis	GCC 7:96
13	D11S16	5	2	0.4	Testis	GCC 9:153
13	D11S16	13	1	0.08	Uterus	CR 51:5632
13	D11S151	4	0	0	Lung	PN 91:5513
13	D11S151	1	0	0	Lung	PN 91:5513
13	D11S151	3	0	0	Lung	PN 91:5513
13	D11S151	11	3	0.27	Pediatric	CR 50:3279
13	D11S151	1	0	0	Testis	GCC 9:153
13	D11S151	4	0	0	Testis	GCC 9:153
13	CAT	18	13	0.72	Bladder	HG 91:455
13	CAT	1	0	0	Kidney	CMB 38:59
13	CAT	16	2	0.12	Kidney	CR 51:1071
13	CAT	6	1	0.17	Kidney	CMB 38:59
13	CAT	7	0	0	Liver	CCG 48:72
13	CAT	6	0	0	Liver	GCC 1:312
13	CAT	8	3	0.38	Lung	PN 86:5099
13	CAT	2	0	0	Lung	PN 86:5099
13	CAT	40	6	0.15	Lung	GCC 10:183
13	CAT	7	1	0.14	Lung	PN 86:5099

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13	CAT	2	1	0.5	Lung	PN 91:5513
13	CAT	7	0	0	Lung	PN 91:5513
13	CAT	10	0	0	Ovary	IJC 54:546
13	CAT	24	6	0.25	Ovary	BRJ 66:103
13	CAT	14	2	0.14	Pediatric	CR 50:3279
13	CAT	4	1	0.25	Stomach	HG 89:445
13	CAT	12	5	0.42	Testis	JU 153:168
13	CAT	1	0	0	Testis	CCG 52:72
13	CAT	3	1	0.33	Testis	CCG 52:72
13	CAT	1	0	0	Testis	CCG 52:72
13	D11S325	3	0	0	Lung	PN 91:5513
13	D11S325	5	0	0	Lung	PN 91:5513
13	D11S325	6	2	0.33	Testis	GCC 9:153
13	D11S325	6	1	0.17	Testis	GCC 9:153
13	D11S325	16	2	0.12	Testis	GCC 7:96
13	D4S414	15	5	0.33	Bladder	BG 91:455
13	D4S414	2	1	0.5	Lung	CR 54:5643
13	D4S414	4	1	0.25	Lung	CR 54:5643
13	D4S414	21	4	0.19	Lung	CR 54:5643
13	B-FSB	16	6	0.38	Bladder	BG 91:455
13	B-FSH	4	0	0	Cervix	BJC 67:71
13	B-FSH	46	9	0.2	Lung	GCC 10:103
13	B-FSH	24	7	0.29	Ovary	BRJ 66:103
13	B-FSH	14	5	0.36	Pediatric	CR 50:3279
13	B-FSH	7	1	0.14	Stomach	HG 89:445
13	D11S905	25	0	0	Esophageal	IJC 69:1
13	D11S905	18	4	0.22	Pediatric	HG 97:163
11.2-12	D11S149	5	0	0	Endocrine	CR 51:1154
11.2-12	D11S149	7	1	0.14	Lung	PN 91:5513
11.2-12	D11S149	1	0	0	Lung	PN 91:5513
11.2-12	D11S149	5	0	0	Lung	PN 91:5513
12	D11S288	10	2	0.2	Cervix	PNAS 91:69
12	D11S1313	48	12	0.25	Lung	GCC 13:40
12	D11S1313	48	12	0.25	Lung	GCC 13:40
Unknown	D11S:907-929	28	15	0.54	Bladder	CR 55:5213
Unknown	Unknown	14	3	0.21	Brain	CR 50:5184
15	Unknown	35	2	0.06	Breast	JNCI 84:50
Unknown	D11SS1318	18	6	0.33	Breast	HMG 4:1889
Unknown	D11SS1323	9	5	0.56	Breast	HMG 4:1889
Unknown	D11SS1338	9	5	0.56	Breast	HMG 4:1889
Unknown	D11SS1760	7	2	0.29	Breast	HMG 4:1889
11	D11S554	22	5	0.23	Cervix	BJC 71:814
Unknown	D11S740	5	0	0	Cervix	GCC 9:119
11	D11S554	22	6	0.27	Endocrine	CR 56:599
15.5	D11S576	25	0	0	Kidney	BJC 69:230
Unknown	D11S-922-904	6	3	0.5	Kidney	GCC 12:76

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15.5	JW1-51	16	4	0.25	Kidney	CR 51:1071
pter-p13	D11S17	6	0	0	Liver	CCG 48:72
13	D11S18	11	1	0.09	Liver	CCG 48:72
13	D11S21	5	0	0	Liver	CCG 48:72
15	HBBC	8	1	0.12	Liver	CCG 48:72
15.3-15.4	D11S1243	57	14	0.25	Lung	GCC 13:40
14	D11S1246	57	17	0.3	Lung	GCC 13:40
15.2-15.3	D11S1250	50	17	0.34	Lung	GCC 13:40
15.4-15.5	D11S1251	66	21	0.32	Lung	GCC 13:40
11.2-12	D11S1252	54	13	0.24	Lung	GCC 13:40
15.4-15.5	D11S1254	39	12	0.31	Lung	GCC 13:40
Unknown	HRAS-INS-HBG	1	1	1	Lung	CR 50:2303
Unknown	HRAS-INS-HBG	27	4	0.15	Lung	CR 50:2303
Unknown	HRAS-INS-HBG	1	0	0	Lung	CR 50:2303
Unknown	HRAS-INS-HBG	13	4	0.31	Lung	CR 50:2303
Unknown	HRAS-INS-HBG	3	0	0	Lung	CR 50:2303
15.5	ST5	4	0	0	Lung	PN 91:5513
15.5	ST5	1	0	0	Lung	PN 91:5513
15.5	ST5	9	0	0	Lung	PN 91:5513
Unknown	D11S922-904	82	4	0.12	Melanoma	CR 56:589
Unknown	Unknown	11	2	0.18	Ovary	IJC 52:575
15	Unknown	5	1	0.2	Ovary	O 5:219
15	Unknown	9	4	0.44	Ovary	O 5:219
Unknown	CALCA-HRAS1-INS-PTH	17	9	0.53	Ovary	GO 55:198
pter-p13	D11S17	17	6	0.35	Ovary	BRJ 66:103
Unknown	D11S554-875-871	18	6	0.33	Ovary	BJC 72:133
Unknown	RAS-CAT-D11S16	34	12	0.35	Ovary	CR 53:2393
15.5	Unknown	3	0	0	Pancreas	CR 54:2761
Unknown	D11S1323	7	2	0.29	Pediatric	HG 97:163
Unknown	D11S1338	14	3	0.21	Pediatric	HG 97:163
Unknown	D11S937	10	1	0.1	Pediatric	HG 97:163
13	WT1	16	8	0.5	Pediatric	HG 97:163
Unknown	Unknown	11	0	0	Prostate	CSurveys 1
Unknown	Unknown	10	0	0	Prostate	PNAS 87:87
Unknown	CALCA-HRAS1-HBG2	15	0	0	Prostate	G 11:530
Unknown	D11S2351	40	16	0.4	Stomach	CR 56:268
Unknown	D11S324	8	3	0.38	Testis	GCC 9:153
Unknown	D11S324	7	3	0.43	Testis	GCC 9:153
Unknown	D11S417	11	3	0.27	Testis	GCC 9:153
Unknown	D11S417	5	3	0.6	Testis	GCC 9:153
Unknown	FSHB	4	0	0	Testis	GCC 9:153
Unknown	FSHB	8	3	0.38	Testis	GCC 9:153
Unknown	FSHB	7	2	0.29	Testis	GCC 7:96
13	WT1	10	5	0.5	Testis	GCC 7:96
Unknown	D11S740	8	1	0.12	Uterus	GCC 9:119
13	WT1	24	0	0	Uterus	CR 54:4294

Chromosome 11 - p Arm

SUM	4917	1151	0.23

Chromosome 11 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
12-13.2	PYGM	12	5	0.42	Breast	CR 54:4586
12-13.3	PYGM-INT2	36	24	0.67	Breast	CR 55:467
12-13.2	PYGM	30	5	0.17	Cervix	PNAS 91:6953
12-13.2	PYGM	3	2	0.67	Endocrine	GCC 12:73
12-13.2	PYGM	16	6	0.38	Endocrine	CR 56:599
12-13.2	PYGM	4	2	0.5	Endocrine	CR 51:1154
12-13.2	PYGM	42	5	0.12	Esophageal	GCC 10:177
12-13.2	PYGM	15	2	0.13	Kidney	CR 51:5817
12-13.2	PYGM	13	0	0	Prostate	G 11:530
12-13.2	PYGM	7	2	0.29	Stomach	HG 89:445
12	CD20	12	3	0.25	Ovary	BJC 67:268
Unknown	PGA	11	0	0	Colon	CCG 48:167
Unknown	PGA	6	1	0.17	Endocrine	CR 51:1154
Unknown	PGA	15	2	0.13	Testis	GCC 7:96
Unknown	PGA	15	2	0.13	Testis	LI 73:606
13	FGF3	40	4	0.1	Breast	CR 54:6270
13	FGF3	16	3	0.19	Ovary	BJC 67:268
13	D11S913	36	0	0	Esophageal	IJC 69:1
13.1	D11S97	25	7	0.28	Cervix	PNAS 91:6953
13.1	D11S97	23	4	0.17	Testis	GCC 13:249
12-13.2	D11S146	6	2	0.33	Endocrine	CR 51:1154
12-13.2	D11S146	15	1	0.07	Kidney	CR 51:5817
12-13.2	D11S146	23	3	0.13	Liver	CR 51:89
12-13.2	D11S146	10	1	0.1	Ovary	BJC 67:268
13	WT-1	14	7	0.5	Bladder	HG 91:455
13	WT-1	13	4	0.31	Breast	CR 54:6270
13	WT-1	20	6	0.3	Cervix	PNAS 91:6953
13	WT-1	52	5	0.1	Lung	GCC 10:183
13	WT-1	21	4	0.19	Lung	CR 54:5643
13	WT-1	2	1	0.5	Lung	CR 54:5643
13	WT-1	4	0	0	Lung	PN 91:5513
13	WT-1	1	0	0	Lung	PN 91:5513
13	WT-1	6	0	0	Lung	PN 91:5513
13	WT-1	4	1	0.25	Lung	CR 54:5643
13	INT2	22	8	0.36	Bladder	CR 55:5213
13	INT2	3	0	0	Breast	CR 53:3804
13	INT2	12	0	0	Breast	CR 50:7184
13	INT2	34	5	0.15	Breast	CR 53:4356
13	INT2	9	1	0.11	Cervix	GCC 9:119
13	INT2	22	1	0.05	Cervix	CR 54:4481
13	INT2	3	1	0.33	Cervix	CR 54:4481
13	INT2	15	0	0	Cervix	CR 49:3598
13	INT2	22	8	0.36	Cervix	PNAS 91:6953
13	INT2	22	7	0.32	Colon	GCC 6:45
13	INT2	5	2	0.4	Endocrine	GCC 12:73
13	INT2	11	3	0.27	Endocrine	CR 51:1154

Chromosome 11 - q Arm

13	INT2	9	0	0	Esophageal	CR 51:2113
13	INT2	13	6	0.46	Head&Neck	CR 54:1152
13	INT2	9	3	0.33	Kidney	CR 51:820
13	INT2	9	3	0.33	Kidney	CR 51:5817
13	INT2	4	1	0.25	Kidney	CR 51:1071
13	INT2	7	1	0.14	Liver	CR 51:4367
13	INT2	11	3	0.27	Lung	PNAS 86:5099
13	INT2	3	1	0.33	Lung	PNAS 86:5099
13	INT2	11	2	0.18	Lung	PNAS 86:5099
13	INT2	24	3	0.12	Lung	CR 52:2478
13	INT2	6	0	0	Ovary	CR 50:2721
13	INT2	21	0	0	Ovary	IJC 54:546
13	INT2	19	1	0.05	Ovary	CR 51:5118
13	INT2	8	2	0.25	Stomach	HG 89:445
13	INT2	18	0	0	Stomach	CR 51:2926
13	INT2	11	1	0.09	Stomach	CR 48:2988
13	INT2	27	4	0.15	Testis	O 9:2245
13	INT2	4	2	0.5	Testis	O 9:2245
13	INT2	3	1	0.33	Testis	CCG 52:72
13	INT2	4	1	0.25	Testis	CCG 52:72
13	INT2	11	2	0.18	Uterus	GCC 9:119
13	INT2	5	1	0.2	Uterus	CR 51:5632
13;2-22	D11S141	8	0	0	Stomach	HG 89:445
13	D11S534	23	6	0.26	Cervix	BJC 71:814
13	D11S534	13	4	0.31	Ovary	Unknown
Unknown	D11S533	38	12	0.32	Cervix	PNAS 91:6953
Unknown	D11S533	21	5	0.24	Endocrine	GCC 13:9
Unknown	D11S533	16	4	0.25	Ovary	GO 55:245
Unknown	D11S911	23	3	0.13	Cervix	CR 56:197
23.3	D11S901	39	13	0.33	Breast	CR 54:4586
23.3	D11S901	33	11	0.33	Cervix	PNAS 91:6953
23.3	D11S901	21	6	0.29	Stomach	CR 56:268
14-23	TYR	2	0	0	Lung	PN 91:5513
14-21	TYR	7	0	0	Lung	PN 91:5513
14-21	TYR	7	1	0.14	Lung	PN 91:5513
14-21	TYR	16	3	0.19	Ovary	BJC 67:268
14-21	TYR	3	2	0.67	Stomach	HG 89:445
22-23	D11S923	36	2	0.06	Esophageal	IJC 69:1
22	D11S35	28	7	0.25	Breast	CR 54:6270
22	D11S35	34	12	0.35	Breast	CR 54:4586
22	D11S35	21	12	0.57	Cervix	PNAS 91:6953
22	D11S35	34	10	0.29	Stomach	CR 56:268
22	D11S35	33	4	0.12	Uterus	CR 54:4294
22	STMY1	12	6	0.5	Colon	GCC 6:45
22	STMY1	11	6	0.55	Ovary	BJC 67:268
22	STMY1	7	2	0.29	Stomach	HG 89:445

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22-23	DRD2	60	23	0.34	Colon	BJC 70:395
Unknown	D11S1341	8	3	0.38	Stomach	CR 56:268
22.3-23.3	D11S144	6	1	0.17	Brain	CR 49:6522
22.3-23.3	D11S144	19	13	0.68	Cervix	PNAS 91:6953
22.3-23.3	D11S144	15	3	0.2	Esophageal	CR 54:2996
22.3-23.3	D11S144	17	5	0.29	Ovary	BJC 67:268
22.3-23.3	D11S144	4	2	0.5	Pancreas	CR 54:2761
22.3-23.3	D11S144	21	4	0.19	Sarcoma	CR 52:2419
22.3-23.3	D11S144	4	0	0	Stomach	HG 89:445
23.3	D11S29	47	15	0.32	Breast	CR 54:6270
23.3	D11S29	1	0	0	Breast	CR 53:3804
23.3	D11S29	25	25	1	Cervix	BJC 71:814
23.3	D11S29	2	1	0.5	Colon	GCC 6:45
23.3	D11S29	12	7	0.58	Melanoma	GCC 7:169
23.3	D11S29	15	7	0.47	Ovary	BJC 67:268
23.3	D11S29	10	6	0.6	Stomach	CR 56:268
23	CD3	7	4	0.57	Colon	GCC 6:45
23.3	CD3	1	0	0	Lung	PN 91:5513
23.3	CD3	9	0	0	Lung	PN 91:5513
23.3	CD3	3	0	0	Lung	PN 91:5513
23.3	CD3	16	7	0.44	Ovary	BJC 67:268
23	CD3	4	2	0.5	Stomach	HG 89:445
23.3	CD3	36	8	0.22	Stomach	CR 56:268
23	D11S528	42	16	0.38	Breast	CR 54:6270
23	D11S528	44	7	0.16	Stomach	CR 56:269
22.3-23	THY1	33	14	0.42	Breast	CR 54:4591
22.3-23	THY1	6	1	0.17	Stomach	HG 89:445
23.3-qter	D11S147	12	8	0.67	Ovary	BJC 67:268
22-23.3	APOC3	35	12	0.34	Breast	CR 54:4586
22-23.3	APOC3	30	19	0.63	Cervix	PNAS 91:6953
22-23.3	APOC3	22	0	0	Pediatric	HG 97:163
Unknown	D11S836	17	6	0.35	Ovary	Unknown
Unknown	D11S934	30	5	0.17	Cervix	CR 56:197
23	ETS1	5	3	0.6	Colon	GCC 6:45
23	ETS1	1	0	0	Lung	PN 91:5513
23	ETS1	4	0	0	Lung	PN 91:5513
23	ETS1	5	0	0	Lung	PN 91:5513
23	ETS1	1	0	0	Testis	CCG 52:72
Unknown	D11S910	22	3	0.14	Head&Neck	CR 54:4756
Unknown	D11S910	31	0	0	Head&Neck	CR 54:4756
Unknown	D11S910	6	3	0.5	Kidney	GCC 12:76
Unknown	D11S910	30	5	0.17	Melanoma	CR 56:589
22.3-23	D11S968	33	11	0.42	Breast	CR 54:4586
22.3-23	D11S968	25	14	0.56	Cervix	PNAS 91:6953
22.3-23	D11S968	5	1	0.2	Kidney	PNAS 92:2854
22.3-23	D11S968	17	1	0.06	Kidney	PNAS 92:2854

Chromosome 11 - q Arm

22.3-23	D11S968	17	1	0.06	Kidney	PNAS 92:2854
Unknown	Unknown	16	1	0.06	Brain	CR 50:5784
13	Unknown	25	1	0.04	Breast	JNCI 84:506
Unknown	D11S485	16	9	0.56	Cervix	PNAS 91:6953
13	Unknown	7	0	0	Endocrine	N 328:524
Unknown	D11S129	7	1	0.14	Endocrine	CR 51:1154
Unknown	D11S1383	5	4	0.8	Endocrine	CR 56:599
Unknown	D11S460	7	3	0.43	Endocrine	GCC 12:73
Unknown	D11S476	2	1	0.5	Endocrine	GCC 12:73
Unknown	D11S527	7	5	0.71	Endocrine	CR 56:599
Unknown	D11S546	4	0	0	Endocrine	GCC 12:73
Unknown	D11S614	22	5	0.23	Endocrine	CR 56:599
Unknown	D11S787	6	4	0.67	Endocrine	CR 56:599
Unknown	D11S873	23	6	0.26	Endocrine	CR 56:599
Unknown	D11S874	13	3	0.23	Endocrine	CR 56:599
Unknown	D11S490	19	9	0.47	Head&Neck	CR 54:1152
13	Unknown	7	0	0	Liver	BJC 67:1007
13	Unknown	10	0	0	Liver	BJC 64:1083
13-23	D11S24	2	0	0	Liver	JJ 81:108
14-22.3	D11S1240	53	12	0.23	Lung	GCC 13:40
13.1-13.4	D11S1253	67	13	0.19	Lung	GCC 13:40
21-23.2	D11S1256	67	21	0.31	Lung	GCC 13:40
14-22.3	D11S1260	20	8	0.4	Lung	GCC 13:40
13.4-14	D11S1261	39	11	0.28	Lung	GCC 13:40
23.2-23.3	D11S1263	65	11	0.17	Lung	GCC 13:40
23.2-23.3	D11S1265	50	14	0.28	Lung	GCC 13:40
14-22.3	D11S1268	30	10	0.33	Lung	GCC 13:40
13-23	D11S24	2	0	0	Lung	PN 84:9252
24	D11S488	17	5	0.29	Ovary	GO 55:245
Unknown	D11S85	15	5	0.33	Ovary	CR 53:2393
13	FOLR1	14	1	0.07	Ovary	BJC 67:268
13	Unknown	8	3	0.38	Pancreas	BJC 65:809
Unknown	D11S1818	38	11	0.29	Stomach	CR 56:268
13-23	D11S24	2	0	0	Stomach	CR 48:2988
13-23	D11S24	1	0	0	Uterus	CR 51:5632
Unknown	D11S420	19	0	0	Uterus	CR 54:4294
SUM		2978	764	0.26		

Chromosome 12 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
12.1	KRAS2	3	0	0	Uterus	CR 51:5632
Unknown	D12S16	16	1	0.06	Brain	CR 50:5784
Unknown	D12S16	12	2	0.17	Breast	CR 50:7184
Unknown	D12S16	23	2	0.09	Breast	CR 53:4356
Unknown	D12S2	16	2	0.12	Cervix	CR 54:4481
Unknown	D12S87	24	2	0.08	Cervix	CR 56:197
Unknown	D12S89	25	2	0.08	Cervix	CR 56:197
12.1	KRAS2	7	0	0	Colon	N 331:273
Unknown	D12S77	18	2	0.11	Endocrine	CR 56:599
Unknown	D12S16	26	1	0.04	Esophageal	CR 54:2996
Unknown	D12S16	7	2	0.29	Esophageal	GCC 10:177
Unknown	D12S62	28	5	0.18	Head&Neck	CR 54:1152
Unknown	D12S98	19	1	0.05	Head&Neck	CR 54:4756
Unknown	D12S98	17	0	0	Head&Neck	CR 54:4756
Unknown	D12S16	10	0	0	Kidney	CR 51:820
Unknown	D12S94-D12S77	5	1	0.2	Kidney	PNAS 92:2854
Unknown	D12S94-D12S77	20	0	0	Kidney	PNAS 92:2854
Unknown	D12S98	6	3	0.5	Kidney	GCC 12:76
Unknown	Unknown	43	8	0.19	Leukemia	B 86:3869
Unknown	Unknown	35	8	0.23	Leukemia	B 86:3869
Unknown	D12S56	44	9	0.2	Leukemia	B 86:3869
Unknown	D12S64	54	7	0.13	Leukemia	B 86:3869
Unknown	D12S69	46	4	0.09	Leukemia	B 86:3869
Unknown	D12S89	82	21	0.26	Leukemia	B 87:3368
Unknown	D12S89	50	11	0.22	Leukemia	B 86:3869
Unknown	D12S91	48	9	0.19	Leukemia	B 86:3869
Unknown	D12S94-D12S77	51	6	0.12	Leukemia	B 86:3869
Unknown	D12S:89-91	50	13	0.26	Leukemia	CR 55:5377
Unknown	D12S16	12	1	0.08	Liver	CR 51:89
12.1	KRAS2	4	0	0	Liver	CCG 48:72
Unknown	D12S16	25	5	0.2	Lung	CR 52:2478
12.1	KRAS2	3	1	0.33	Lung	PN 84:9252
Unknown	D12S98	19	0	0	Melanoma	CR 56:589
12.1	KRAS2	2	0	0	Neuroblastoma	CR 49:1095
11.3-12.3	A2M	10	1	0.1	Ovary	IJC 54:546
Unknown	D12S16	8	3	0.38	Ovary	CR 51:5118
12-PTPR	FBVWF	16	1	0.06	Ovary	BJC 69:429
12.1	KRAS2	7	0	0	Ovary	CR 50:2724
Unknown	PRB1	23	2	0.09	Ovary	CR 53:2393
Unknown	D12S16	9	1	0.11	Prostate	G 11:530
12.1	KRAS2	4	1	0.25	Stomach	CR 48:2988
12.1	KRAS2	7	0	0	Testis	GCC 13:249
Unknown	PRB1-PRB4	11	2	0.18	Testis	LI 73:606
Unknown	D12S61	14	1	0.07	Uterus	CR 54:4294
12.1	KRAS2	3	0	0	Uterus	CR 51:5632

Chromosome 12 - p Arm

SUM		959	141	0.15

Chromosome 12 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	IGF1	11	1	0.09	Uterus	CR 54:4294
Unknown	Unknown	14	1	0.07	Brain	CR 50:5784
Unknown	D12S17	19	1	0.05	Breast	CR 50:7184
14-24.1	D12S7	35	2	0.06	Breast	GCC 2:191
Unknown	D12S17	8	1	0.12	Cervix	GCC 9:119
Unknown	D12S7	31	1	0.03	Cervix	CR 54:4481
Unknown	D12S78	31	6	0.19	Cervix	CR 56:197
Unknown	D12S83	22	1	0.05	Cervix	CR 56:197
Unknown	D12S17	19	1	0.05	Colon	CCG 48:167
Unknown	D12S17	17	4	0.24	Colon	IJC 53:382
14-24.1	D12S7	22	3	0.14	Colon	N 331:273
14-qter	D12S8	24	4	0.17	Colon	N 331:273
24.3-qter	D12S11	13	0	0	Endocrine	N 328:524
Unknown	D12S392	16	1	0.06	Endocrine	CR 56:599
Unknown	D12S43	23	0	0	Endocrine	GCC 13:9
Unknown	D12S14	18	3	0.17	Esophageal	CR 54:2996
Unknown	D12S17	9	1	0.11	Esophageal	CR 51:2713
Unknown	D12S17	34	3	0.09	Esophageal	GCC 10:177
Unknown	D12S17	23	2	0.09	Esophageal	CR 54:2996
Unknown	D12S60	24	6	0.25	Head&Neck	CR 54:1152
Unknown	D12S86	24	4	0.17	Head&Neck	CR 54:4756
Unknown	D12S86	18	0	0	Head&Neck	CR 54:4756
Unknown	D12S17	24	0	0	Kidney	CR 51:820
Unknown	D12S86	6	3	0.5	Kidney	GCC 12:76
Unknown	D12S97-D12S86	19	0	0	Kidney	PNAS 92:2854
Unknown	D12S97-D12S86	6	0	0	Kidney	PNAS 92:2854
24.3-qter	Unknown	12	1	0.08	Liver	BJC 64:1083
24.3-qter	Unknown	7	0	0	Liver	BJC 67:1007
Unknown	D12S17	14	1	0.07	Liver	CR 51:99
Unknown	D12S17	15	1	0.07	Liver	JJCR 81:108
Unknown	D12S17	29	4	0.14	Lung	CR 52:2478
Unknown	D12S86	23	0	0	Melanoma	CR 56:589
Unknown	D12S17	25	6	0.24	Ovary	CR 53:2393
Unknown	D12S17	15	5	0.33	Ovary	CR 51:5118
Unknown	D12S60	15	2	0.13	Ovary	BJC 69:425
22-24.2	PAH	26	2	0.08	Ovary	IJC 54:546
24.3-qter	Unknown	13	0	0	Pancreas	BJC 65:809
24.3-qter	Unknown	6	3	0.5	Pancreas	CR 54:2761
Unknown	D12S17	6	0	0	Pancreas	CR 54:2761
14-24.1	D12S7	17	1	0.06	Prostate	G 11:530
Unknown	D12S17	26	5	0.19	Sarcoma	CR 52:2419
CEN-q14	D12S4	5	1	0.2	Sarcoma	CR 52:2419
2.4-ter	Unknown	11	6	0.55	Stomach	BJC 59:750
24.3-qter	D12S11	32	5	0.16	Stomach	HG 92:244
Unknown	D12S17	41	11	0.27	Stomach	CR 51:2926
12-13.2	COL2A1	11	0	0	Testis	GCC 13:249

Chromosome 12 - q Arm

24.3-qter	D12S11	30	0	0	Testis	GCC 13:249
Unknown	D12S12	15	7	0.47	Testis	O 9:2245
Unknown	D12S14	19	3	0.16	Testis	O 9:2245
Unknown	D12S15	14	1	0.07	Testis	O 9:2245
Unknown	D12S17	26	7	0.27	Testis	O 9:2245
CEN-q14	D12S4	23	4	0.17	Testis	O 9:2245
Unknown	D12S6	17	7	0.41	Testis	O 9:2245
14-24.1	D12S7	6	1	0.17	Testis	LI 73:606
14-24.1	D12S7	15	0	0	Testis	GCC 13:249
Unknown	D12S7	1	0	0	Testis	CCG 52:72
Unknown	D12S7	3	0	0	Testis	CCG 52:72
Unknown	D12S7	1	0	0	Testis	CCG 52:72
Unknown	D12S7	19	8	0.42	Testis	O 9:2245
14-qter	D12S8	8	1	0.12	Testis	O 9:2245
Unknown	D12S17	23	4	0.17	Uterus	GCC 9:119
Unknown	D12S60	17	1	0.06	Uterus	CR 54:4294
Unknown	IGF1	11	1	0.09	Uterus	CR 54:4294
SUM		1096	147	0.13		

Chromosome 13 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
12	D13S36	19	5	0.26	Ovary	IJC 54:546
12	D13S36	19	3	0.16	Ovary	IJC 52:575
12.3	D13S11	9	3	0.33	Ovary	IJC 54:546
12.3	D13S11	6	5	0.83	Sarcoma	CGC 53:45
Unknown	D13S115	13	6	0.46	Head&Neck	CR 54:1152
Unknown	D13S115	16	2	0.12	Ovary	BJC 69:429
Unknown	D13S221	28	7	0.25	Bladder	Unknown
Unknown	D13S221	39	17	0.44	Breast	GCC 13:291
12.3	D13S6	4	2	0.5	Breast	PNAS 84:2372
12.3	D13S6	13	5	0.38	Colon	IJC 53:382
12.3	D13S6	1	0	0	Colon	CCG 48:167
12.3	D13S6	8	2	0.25	Ovary	IJC 54:546
12.3	D13S6	9	0	0	Stomach	G 2:189
12.3	D13S6	7	2	0.29	Uterus	CR 51:5632
Unknown	D13S289	35	17	0.49	Breast	GCC 13:291
12	FLT1	7	0	0	Brain	CR 54:1397
12	FLT1	9	3	0.33	Brain	CR 54:1397
12	FLT1	18	6	0.33	Ovary	CR 54:605
12	FLT1	5	1	0.2	Ovary	BJC 69:429
12.3	D13S33	21	4	0.19	Ovary	IJC 54:546
12.3	D13S33	23	6	0.26	Ovary	IJC 52:575
12	D13S260	43	13	0.3	Breast	GCC 13:291
13	D13S1	94	26	0.28	Bladder	O 6 2305
14-12	D13S1	34	7	0.21	Breast	GE 5:554
13	D13S1	8	3	0.38	Breast	PNAS 84:2372
13	D13S1	13	4	0.31	Breast	GCC 2:191
13	D13S1	7	2	0.29	Cervix	CR 49:3598
14-12	D13S1	11	1	0.09	Colon	JNCI 84:1100
13	D13S1	15	7	0.47	Colon	IJC 53:382
12	D13S1	12	1	0.08	Colon	CCG 48:167
13	D13S1	14	4	0.29	Esophageal	CR 54:2996
13	D13S1	10	2	0.2	Kidney	CR 51:1071
13	D13S1	25	5	0.2	Liver	JJCR 84:893
14-12	D13S1	15	5	0.33	Liver	CR 54:281
14-12	D13S1	5	2	0.4	Liver	CCG 48:72
12	D13S1	9	0	0	Liver	JJCR 81:108
14-12	D13S1	9	6	0.67	Liver	CR 51:4967
13	D13S1	19	8	0.42	Lung	PN 84:9252
14-12	D13S1	8	7	0.88	Lung	CR 49:5130
12	D13S1	1	0	0	Lung	PN 84:9252
13	D13S1	5	0	0	Neuroblastoma	CR 49:1095
13	D13S1	15	2	0.13	Ovary	IJC 54:546
13	D13S1	12	9	0.75	Sarcoma	CR 52:2419
13	D13S1	6	0	0	Stomach	HG 89:445
14-12	D13S1	10	1	0.1	Stomach	CR 48:2988

Chromosome 13 - q Arm

14-12	D13S1	11	1	0.09	Testis	LI 73:606
13	D13S1	3	0	0	Testis	CCG 52:72
13	D13S1	3	1	0.33	Testis	CCG 52:72
13	D13S1	1	0	0	Testis	CCG 52:72
13	D13S1	8	1	0.12	Uterus	CR 51:5632
12	D13S267	32	16	0.5	Breast	GCC 13:291
14	D13S218	140	33	0.24	Leukemia	CR 55:2044
12	D13S263	45	20	0.44	Breast	GCC 13:291
14	D13S22	17	5	0.29	Breast	GE 5:554
14	D13S22	11	3	0.27	Breast	GE 5:554
14	D13S22	12	0	0	Pediatric	CR 50:3279
14	D13S22	8	7	0.88	Sarcoma	GCC 53:44
14	D13S153	42	15	0.36	Breast	GCC 13:291
14.3	D13S133	18	10	0.56	Head&Neck	CR 54:1152
14.3	D13S133	6	3	0.5	Kidney	GCC 12:76
14.3	D13S133	140	5	0.04	Leukemia	CR 55:2044
14.3	D13S133	11	0	0	Ovary	CR 54:605
14.3	D13S133	18	11	0.61	Ovary	CR 54:605
14.3	D13S133	21	7	0.33	Prostate	HUPATH 27:28
14.3-21.1	D13S31	29	9	0.31	Ovary	IJC 52:525
14.3-21	D13S31	26	6	0.23	Ovary	IJC 54:546
14	RB	94	28	0.3	Bladder	O 6:2305
14	RB	9	4	0.44	Brain	O 6:445
14	RB	20	3	0.15	Breast	AJP 140:215
14	RB	38	6	0.16	Breast	CR 53:4356
14	RB	14	5	0.36	Breast	JNCI 84:506
14	RB	10	4	0.4	Breast	GCC 4:113
14	RB	32	12	0.38	Breast	GE 5:554
14	RB	37	12	0.32	Breast	GCC 4:113
14	RB	90	23	0.26	Breast	CR 52:2991
14	RB	14	0	0	Cervix	BJC 67:71
14	RB	27	9	0.33	Colon	CR 52:743
14	RB	25	12	0.48	Colon	IJC 53:382
14.1	RB	156	18	0.12	Colon	IJC 64:475
14	RB	39	10	0.26	Colon	GAST 104:163
14	RB	8	0	0	Colon	JNCI 84:1100
14	RB	6	0	0	Colon	JNCI 84:1100
14	RB	42	0	0	Endocrine	C 74:693
14	RB	29	17	0.59	Esophageal	C 73:2472
14	RB	40	19	0.47	Esophageal	CR 51:5766
14	RB	8	1	0.12	Esophageal	CR 51:2113
14	RB	16	5	0.31	Esophageal	CR 54:2996
14	RB	50	24	0.48	Esophageal	CR 52:6525
14	RB	29	17	0.59	Head&Neck	C 73:2472
14	RB	11	4	0.36	Liver	CR 54:281
14	RB	11	3	0.27	Liver	CR 51:4367

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14	RB	9	1	0.11	Liver	CR 51:4367
14	RB	67	13	0.19	Lung	O 8:1913
14	RB	16	0	0	Lung	O 9:39
14	RB	7	2	0.29	Lung	CR 54:5643
14	RB	20	12	0.6	Lung	O 8:1913
14	RB	8	0	0	Lung	S 243:353
14	RB	3	2	0.67	Lung	CL 71:67
14	RB	8	6	0.75	Lung	O 9:39
14	RB	76	28	0.37	Lung	O 8:1913
14	RB	27	14	0.52	Lung	CR 54:5643
14	RB	59	22	0.37	Lung	O 10:937
14	RB	5	4	0.8	Lung	CR 54:5643
14	RB	2	1	0.5	Lung	CL 71:67
14	RB	7	1	0.14	Ovary	GO 55:245
14	RB	13	8	0.62	Ovary	IJC 58:663
14	RB	31	23	0.74	Ovary	CR 54:610
14	RB	39	13	0.33	Ovary	IJC 54:546
14.1	RB	17	2	0.12	Ovary	CR 54:610
14	RB	33	9	0.27	Ovary	IJC 52:575
14	RB	48	25	0.52	Ovary	CR 54:610
14	RB	9	0	0	Pediatric	CR 50:3279
14	RB	15	3	0.23	Prostate	PNAS 87:8751
14.1	RB	9	6	0.67	Prostate	BJU 73:390
14	RB	19	7	0.37	Prostate	HUPATH 27:28
14	RB	40	24	0.6	Prostate	BJC 70:1252
14	RB	7	5	0.71	Sarcoma	CR 52:2419
14	RB	13	4	0.31	Stomach	LI 74:835
14	RB	31	12	0.39	Testis	O 9:2245
Unknown	D13S155	6	3	0.5	Kidney	GCC 12:76
Unknown	D13S155	52	3	0.09	Melanoma	CR 56:589
14.1	D13S118	21	7	0.33	Prostate	HUPATH 27:28
21.1-21.2	D13S26	27	17	0.63	Ovary	GO 47:137
21-qter	D13S12	7	1	0.14	Liver	PNAS 86:8852
21-qter	D13S12	4	4	1	Sarcoma	GGC 53:45
22	D13S2	94	26	0.28	Bladder	O 6:2305
Unknown	D13S2	6	1	0.17	Breast	GCC 2:191
22	D13S2	7	3	0.43	Breast	PNAS 84:2372
22	D13S2	2	0	0	Cervix	CR 49:3598
22	D13S2	4	1	0.25	Cervix	CR 54:4481
22	D13S2	10	3	0.3	Colon	IJC 55:382
22	D13S2	8	0	0	Colon	CCG 48:167
22	D13S2	4	1	0.25	Colon	CCG 48:167
22	D13S2	17	7	0.41	Esophageal	CR 54:2996
22	D13S2	6	2	0.33	Kidney	CR 51:1071
22	D13S2	6	4	0.67	Liver	CCG 48:72
22	D13S2	13	3	0.23	Liver	CR 51:89

Chromosome 13 - q Arm

Unknown	D13S2	13	0	0	Liver	JJCR 81:108
22	D13S2	21	12	0.57	Lung	PN 84:9252
22	D13S2	12	2	0.17	Lung	JJCR 80:924
Unknown	D13S2	9	7	0.78	Lung	CR 49:5130
22	D13S2	7	1	0.14	Neuroblastoma	CR 49:1095
Unknown	D13S2	10	3	0.3	Ovary	IJC 54:546
22	D13S2	8	6	0.75	Sarcoma	CR 52:2419
22	D13S2	10	3	0.3	Stomach	CR 52:3099
22	D13S2	9	1	0.11	Stomach	HG 92:244
22	D13S2	11	2	0.18	Stomach	CR 48:2988
22	D13S2	6	4	0.67	Stomach	G 2:180
Unknown	D13S2	7	1	0.14	Stomach	HG 89/445
Unknown	D13S2	14	4	0.29	Testis	O 9:2245
22	D13S2	4	1	0.25	Uterus	CR 51:5632
22-31	D13S170	47	11	0.23	Breast	GCC 13:291
22-31	D13S170	29	11	0.38	Head&Neck	CR 54:4756
22-31	D13S170	20	0	0	Head&Neck	CR 54:4756
31	D13S4	1	1	1	Breast	GCC 2:191
Unknown	D13S4	26	3	0.12	Breast	GE 5:554
Unknown	D13S4	5	2	0.4	Breast	PNAS 84:2372
Unknown	D13S4	10	0	0	Cervix	CR 49:3598
31	D13S4	8	0	0	Colon	JNCI 84:1100
Unknown	D13S4	1	0	0	Colon	CCG 48:167
Unknown	D13S4	19	12	0.63	Colon	IJC 53:302
Unknown	D13S4	12	4	0.33	Esophageal	CR 54:2996
Unknown	D13S4	4	0	0	Liver	JJCR 81:108
31	D13S4	19	10	0.53	Lung	PN 84:9252
31	D13S4	16	3	0.19	Lung	JJCR 80:924
Unknown	D13S4	5	5	1	Lung	CR 49:5130
31	D13S4	8	0	0	Neuroblastoma	CR 49:1095
Unknown	D13S4	15	11	0.73	Sarcoma	CR 52:2419
31	D13S4	14	3	0.21	Stomach	HG 92:244
Unknown	D13S4	11	2	0.18	Stomach	G 2:180
Unknown	D13S4	17	2	0.12	Stomach	CR 48:2988
Unknown	D13S4	12	0	0	Uterus	CR 51:5632
22-34	D13S5	26	6	0.23	Breast	GE 5:554
21.3-32	D13S5	4	1	0.25	Breast	PNAS 84:2372
21.3-32	D13S5	15	4	0.27	Colon	IJC 53:302
21.3-32	D13S5	4	0	0	Colon	CCG 48:167
22-34	D13S5	1	0	0	Colon	JNCI 84:1100
22-34	D13S5	22	9	0.41	Ovary	IJC 54:546
21.3-32	D13S5	10	4	0.4	Stomach	G 2:180
22-34	D13S5	7	1	0.14	Stomach	G 2:180
21.3-32	D13S5	5	0	0	Uterus	CR 51:5632
22-34	D13S5	3	0	0	Uterus	CR 51:5632

Chromosome 13 - q Arm

21	D13S71	15	2	0.13	Brain	CR 54:1397
21	D13S71	7	0	0	Brain	CR 54:1397
32-34	D13S128	34	12	0.35	Ovary	CR 54:605
34	D13S34	12	5	0.42	Ovary	IJC 52:575
34	D13S34	15	7	0.47	Ovary	IJC 54:546
34	D13S32	28	11	0.39	Ovary	IJC 54:546
34	D13S32	26	12	0.46	Ovary	IJC 52:575
22-31	D13S173	39	7	0.18	Breast	GCC 13:291
34	D13S3	94	26	0.28	Bladder	O 6:2305
Unknown	D13S3	20	3	0.15	Breast	GCC 2:191
34	D13S3	26	4	0.15	Breast	GE 5:554
34	D13S3	7	2	0.29	Breast	PNAS 84:2372
33-34	D13S3	27	3	0.11	Cervix	CR 54:4601
34	D13S3	18	4	0.22	Cervix	CR 49:3598
34	D13S3	15	6	0.4	Colon	IJC 53:382
Unknown	D13S3	6	0	0	Colon	JNCI 84:1100
Unknown	D13S3	4	0	0	Liver	JJCR 81:108
33-34	D13S3	2	1	0.5	Liver	CCG 48:72
34	D13S3	8	4	0.5	Liver	CR 51:4367
34	D13S3	9	4	0.44	Lung	PNAS 86:5099
Unknown	D13S3	23	7	0.3	Lung	PN 84:9252
34	D13S3	11	10	0.91	Lung	CR 49:5130
34	D13S3	24	9	0.38	Lung	PN 84:9252
34	D13S3	9	4	0.44	Lung	PNAS 86:5099
34	D13S3	7	1	0.14	Neuroblastoma	CR 49:1095
34	D13S3	21	3	0.14	Ovary	IJC 52:575
34	D13S3	19	4	0.21	Ovary	IJC 54:546
Unknown	D13S3	9	4	0.44	Sarcoma	CR 52:2419
34	D13S3	5	0	0	Stomach	HG 89:445
34	D13S3	20	5	0.25	Stomach	G 2:180
33-34	D13S3	9	1	0.11	Stomach	HG 92:244
Unknown	D13S3	19	5	0.26	Stomach	G 2:180
33-34	D13S3	17	2	0.12	Stomach	CR 48:2988
Unknown	D13S3	1	0	0	Testis	CCG 52:72
34	D13S3	20	8	0.4	Testis	O 9:2245
Unknown	D13S3	4	0	0	Testis	CCG 52:72
Unknown	D13S3	2	0	0	Testis	CCG 52:72
34	D13S3	7	1	0.14	Uterus	CR 51:5632
34	D13S35	17	2	0.12	Ovary	IJC 54:546
34	D13S35	18	2	0.11	Ovary	IJC 52:575
Unknown	D13S52	33	7	0.21	Breast	CR 50:7184
Unknown	D13S52	132	34	0.26	Breast	CR 51:5794
Unknown	D13S52	53	23	0.43	Esophageal	GCC 10:177
Unknown	D13S52	16	3	0.19	Esophageal	CR 51:2113
Unknown	D13S52	22	10	0.45	Esophageal	CR 54:2996
Unknown	D13S52	20	3	0.15	Kidney	CR 51:820

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Unknown	D13S52	26	4	0.15	Liver	CR 51:89
Unknown	D13S52	2	1	0.5	Lung	CR 52:2478
Unknown	D13S52	9	5	0.56	Lung	CR 52:2478
Unknown	D13S52	26	5	0.19	Lung	CR 52:2478
Unknown	D13S52	1	1	1	Lung	CR 52:2478
Unknown	D13S52	27	6	0.22	Ovary	CR 51:5118
34	F7	11	2	0.18	Ovary	IJC 54:546
34	F7	11	2	0.18	Ovary	IJC 54:546
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	1	1	1	Bladder	CR 55:4830
Unknown	D13S30	3	0	0	Bladder	CR 51:5405
Unknown	D13S-133-170	30	15	0.5	Bladder	CR 55:5213
Unknown	Unknown	7	1	0.14	Brain	CR 49:6572
Unknown	Unknown	14	2	0.14	Brain	CR 50:5704
32	D13S193	13	2	0.15	Brain	CR 54:1397
32	D13S193	13	0	0	Brain	CR 54:1397
Unknown	RB1-D13S4-D13S63	7	0	0	Brain	CGC 73:122
Unknown	RB1-D13S4-D13S63	19	2	0.11	Brain	CGC 73:122
Unknown	RB1-D13S4-D13S63	10	0	0	Brain	CGC 73:122
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	1	1	1	Breast	CR 55:4830
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	33	28	0.85	Breast	CR 55:4830
Unknown	D13S7	2	1	0.5	Breast	PNAS 84:2372
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	1	1	1	Cervix	CR 55:4830
Unknown		6	0	0	Colon	JNCI 84:1100
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	1	1	1	Colon	CR 55:4830
Unknown	D13S10	5	0	0	Colon	CCG 48:167
Unknown	D13S37	21	1	0.05	Colon	CCG 48:167
Unknown	FSD	19	0	0	Colon	CCG 48:167
Unknown	D13S168	18	2	0.11	Endocrine	CR 56:599
Unknown	D13S174-D13S173	20	1	0.05	Kidney	PNAS 92:2854
Unknown	D13S174-D13S173	5	0	0	Kidney	PNAS 92:2854
Unknown	D13S-156-158-164-217-221	24	5	0.42	Leukemia	CR 55:5377
Unknown	Unknown	11	0	0	Liver	BJC 64:1083
Unknown	Unknown	5	0	0	Liver	BJC 67:1007
Unknown	14.2	7	0	0	Liver	BJC 67:1007
p13-q11	D13S11	1	1	1	Liver	PNAS 86:8852
Unknown	Unknown	24	18	0.75	Lung	CR 54:2322
33-qter	Unknown	3	1	0.33	Lung	PN 86:5099
33-qter	Unknown	9	4	0.44	Lung	PN 86:5099

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33-qter	Unknown	9	4	0.44	Lung	PN 86:5099
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	6	5	0.83	Ovary	CR 55:4830
Unknown	D13S3-2-1-RB1	32	18	0.56	Ovary	CR 53:2393
Unknown	Unknown	7	0	0	Pancreas	BJC 65:809
Unknown	14.2	10	0	0	Pancreas	BJC 65:809
Unknown	Unknown	13	3	0.23	Prostate	CSurveys 11:
Unknown	BRAC2 (D13S:263-219-220-267-171-260-217)	7	6	0.86	Prostate	CR 55:4830
Unknown	D13S3-D13S5	11	1	0.09	Prostate	G 11:530
Unknown	D13S103	32	5	0.16	Stomach	HG 92:244
Unknown	D13S409	14	2	0.14	Stomach	CR 55:1933
Unknown	Unknown	15	3	0.2	Testis	G 5:134
Unknown	D13S103	9	1	0.11	Testis	GCC 13:249
Unknown	D13S70	13	3	0.23	Testis	GCC 13:249
Unknown	D13S120	15	0	0	Uterus	CR 54:4294
Unknown	D13S122	18	2	0.11	Uterus	CR 54:4294
SUM		5208	1509	0.29		

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D14S22	24	2	0.08	Esophageal	CR 54 2996
SUM		24	2	0.08		

Chromosome 14 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	TCRD	31	6	0.19	Uterus	CR 54:4294
Unknown	D14S:267-268-51	30	21	0.7	Bladder	CR 55:5213
Unknown	Unknown	19	3	0.16	Brain	CR 50:5704
32	D14S13	14	1	0.07	Brain	CR 49:6572
32.1-32.2	D14S13	26	1	0.04	Brain	CR 55:4696
32.1-32.2	D14S13	26	1	0.04	Brain	CR 55:4696
32	D14S16	26	1	0.04	Brain	CR 55:4696
32	D14S16	26	1	0.04	Brain	CR 55:4696
32.32-.33	D14S23	26	0	0	Brain	CR 55:4696
32.32-.33	D14S23	26	0	0	Brain	CR 55:4696
24.3	D14S43	26	5	0.19	Brain	CR 55:4696
24.3	D14S43	26	5	0.19	Brain	CR 55:4696
32.1-32.2	D14S45	26	1	0.04	Brain	CR 55:4696
32.1-32.2	D14S45	26	1	0.04	Brain	CR 55:4696
24.3-31	D14S48	26	8	0.31	Brain	CR 55:4696
24.3-31	D14S48	26	8	0.31	Brain	CR 55:4696
32.1-32.2	D14S51	26	3	0.12	Brain	CR 55:4696
32.1-32.2	D14S51	26	3	0.12	Brain	CR 55:4696
12.0-13.0	D14S54	26	2	0.08	Brain	CR 55:4696
12.0-13.0	D14S54	26	2	0.08	Brain	CR 55:4696
23-31	D14S59	26	10	0.38	Brain	CR 55:4696
23-31	D14S59	26	10	0.38	Brain	CR 55:4696
12.0-13.0	D14S70	26	8	0.31	Brain	CR 55:4696
12.0-13.0	D14S70	26	8	0.31	Brain	CR 55:4696
24.3-31	D14S76	26	6	0.23	Brain	CR 55:4696
24.3-31	D14S76	26	6	0.23	Brain	CR 55:4696
12	D14S80	26	7	0.27	Brain	CR 55:4696
12	D14S80	26	7	0.27	Brain	CR 55:4696
31	D14S81	26	7	0.27	Brain	CR 55:4696
31	D14S81	26	7	0.27	Brain	CR 55:4696
32.3	IGH	26	9	0.35	Brain	CR 55:4696
32.3	IGH	26	9	0.35	Brain	CR 55:4696
32	D14S13	60	7	0.12	Breast	CR 53:4356
32	D14S13	29	7	0.24	Breast	GCC 2:191
32	D14S13	47	6	0.13	Breast	CR 50:7184
32	D14S16	17	2	0.12	Breast	GCC 2:191
32.3	IGH	6	2	0.33	Breast	CR 53:3804
32.32-.33	D14S1	10	2	0.2	Cervix	CR 49:3598
32.33	D14S20	10	1	0.1	Cervix	CR 54:1481
Unknown	D14S3	7	0	0	Cervix	GCC 9:119
32.1	AACT	26	6	0.23	Colon	O 8:671
32.32-32.33	AKTI	10	4	0.4	Colon	O 8:671
32.32-.33	D14S1	42	14	0.33	Colon	O 8:671
32.33	D14S1	28	12	0.43	Colon	IJC 53:382
32	D14S13	35	14	0.4	Colon	IJC 53:382
Unknown	D14S16	17	2	0.12	Colon	CCG 48:167

Chromosome 14 - q Arm

32	D14S16	14	7	0.5	Colon	IJC 53:382
32	D14S16	37	18	0.49	Colon	O 8:671
32.32-.33	D14S17	12	5	0.42	Colon	IJC 53:382
32.32-.33	D14S17	20	7	0.35	Colon	O 8:671
32.1-32.32	D14S18	1	1	1	Colon	IJC 53:382
32.32-32.33	D14S19	39	22	0.56	Colon	O 8:671
32.33	D14S19	14	4	0.29	Colon	IJC 53:382
32.33	D14S20	20	10	0.5	Colon	O 8:671
32.1-32.32	D14S21	2	2	1	Colon	IJC 53:382
32.1-32.32	D14S21	23	6	0.26	Colon	O 8:671
32.32-.33	D14S23	23	9	0.39	Colon	IJC 53:382
32.32-.33	D14S23	42	21	0.5	Colon	O 8:671
32.3	IGH	47	26	0.55	Colon	O 8:671
32.1	PI	6	0	0	Colon	O 8:671
Unknown	D14S174	31	0	0	Endocrine	GCC 13:9
32.1-32.2	D14S45	23	0	0	Endocrine	CR 56:599
32	D14S13	23	4	0.17	Esophageal	CR 51:2113
32	D14S13	64	9	0.14	Esophageal	GCC 10:177
32	D14S13	26	4	0.15	Esophageal	CR 54:2996
Unknown	D14S51	23	9	0.39	Head&Neck	CR 54:1152
Unknown	D14S73	20	1	0.05	Head&Neck	CR 54:4756
Unknown	D14S73	18	1	0.06	Head&Neck	CR 54:4756
32	D14S13	36	3	0.08	Kidney	CR 51:820
Unknown	D14S65-D14S81	6	1	0.17	Kidney	PNAS 92:28
Unknown	D14S65-D14S81	22	5	0.23	Kidney	PNAS 92:28
Unknown	Unknown	10	0	0	Liver	BJC 64:108
Unknown	Unknown	5	0	0	Liver	BJC 67:100
32.32-.33	D14S1	3	0	0	Liver	CCG 48:72
32.32-.33	D14S1	17	6	0.35	Liver	JJCR 81:10
32	D14S13	46	5	0.11	Liver	CR 51:89
Unknown	D14S15	2	0	0	Liver	PNAS 86:185
32.32-.33	D14S1	1	1	1	Lung	CR 54:5643
32.32-.33	D14S1	17	7	0.41	Lung	CR 54:5643
32.32-.33	D14S1	8	1	0.12	Lung	CR 54:5643
32.32-.33	D14S1	23	2	0.09	Lung	PN 84:9852
32	D14S13	50	6	0.12	Lung	CR 52:2478
32.33	D14S1	22	7	0.32	Neuroblastoma	O 7:1185
32.32-.33	D14S1	16	8	0.5	Neuroblastoma	CR 49:1095
32.32-.33	D14S1	19	4	0.21	Neuroblastoma	O 7:1185
32.1-32.2	D14S13	24	5	0.21	Neuroblastoma	O 7:1185
32	D14S16	13	8	0.62	Neuroblastoma	O 7:1185
32.32-.33	D14S17	18	1	0.06	Neuroblastoma	O 7:1185

Chromosome 14 - q Arm

32.32-32.33	D14S19	20	4	0.2	Neuroblastom a	O 7:1185
32.1-32.32	D14S21	18	1	0.06	Neuroblastom a	O 7:1185
11.2-13	MYH6	17	0	0	Neuroblastom a	O 7:1185
32.32-.33	D14S1	26	2	0.08	Ovary	IJC 54:546
32	D14S13	28	5	0.18	Ovary	CR 51:5118
32	D14S16	15	7	0.47	Ovary	CR 53:2393
32.33	D14S20	9	3	0.33	Ovary	O 7:1059
Unknown	D14S34	13	7	0.54	Ovary	BJC 69:429
24.3-31	D14S48	9	3	0.33	Ovary	BJC 69:429
Unknown	D14S49	20	5	0.25	Ovary	BJC 69:429
Unknown	D14S50	10	3	0.3	Ovary	BJC 69:429
Unknown	D14S51	17	4	0.24	Ovary	BJC 69:429
Unknown	Unknown	6	0	0	Pancreas	BJC 65:809
32	D14S13	4	0	0	Pancreas	CR 54:2761
32.32-.33	D14S1	7	0	0	Prostate	G 11:530
32.32-.33	D14S1	7	0	0	Sarcoma	CR 52:2419
32	D14S13	29	1	0.03	Sarcoma	CR 52:2419
32.32-.33	D14S1	16	1	0.06	Stomach	CR 48:2988
Unknown	D14S44	32	5	0.16	Stomach	HG 92:244
32.33	D14S20	8	1	0.12	Testis	O 9:2245
Unknown	D14S44	21	2	0.1	Testis	GCC 13:249
32.32-.33	D14S1	10	0	0	Uterus	CR 51:5632
Unknown	D14S53	12	1	0.08	Uterus	GCC 9:119
24.3-31	D14S76	28	3	0.11	Uterus	CR 54:4294
11.2-13	MYH6	18	2	0.11	Uterus	CR 54:4294
Unknown	TCRD	31	6	0.19	Uterus	CR 54:4294
SUM		2442	542	0.22		

Chromosome 15 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D15S25	26	4	0.15	Esophageal	CR 54:2996
Unknown	D15S25	9	0	0	Colon	CCG 48:167
Unknown	D15S25	26	4	0.15	Esophageal	CR 54:2996
SUM		35	4	0.11		

Chromosome 15 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
26.1	PES	36	5	0.14	Uterus	CR 54:4293
Unknown	Unknown	18	3	0.17	Brain	CR 50:5784
Unknown	D15S27	7	1	0.14	Brain	CR 49:6572
14-21	D15S1	28	1	0.04	Breast	GCC 2:191
11-12.0	D15S11	34	3	0.09	Breast	CR 5314356
pter-q13	D15S24	2	1	0.5	Breast	CR 53:3804
Unknown	D15S28	12	2	0.17	Breast	CR 50:7184
Unknown	D15S29	16	4	0.25	Breast	GCC 2:191
14-21	D15S1	6	0	0	Cervix	CR 49:3598
pter-q13	D15S24	23	0	0	Cervix	CR 54:4481
14-21	D15S1	6	1	0.17	Colon	N 351:273
Unknown	ACTC	36	6	0.17	Endocrine	CR 56:599
Unknown	CYP19	33	5	0.15	Endocrine	CR 56:599
14-21	D15S1	5	4	0.8	Endocrine	CR 56:599
Unknown	D15S100	31	5	0.16	Endocrine	CR 56:599
Unknown	D15S107	8	6	0.75	Endocrine	CR 56:599
Unknown	D15S108	8	3	0.38	Endocrine	CR 56:599
Unknown	D15S114	4	4	1	Endocrine	CR 56:599
Unknown	D15S116	21	7	0.33	Endocrine	CR 56:599
Unknown	D15S118	16	5	0.31	Endocrine	CR 56:599
Unknown	D15S125	24	5	0.21	Endocrine	CR 56:599
Unknown	D15S127	10	7	0.7	Endocrine	CR 56:599
Unknown	D15S144	9	7	0.78	Endocrine	CR 56:599
Unknown	D15S165	32	7	0.22	Endocrine	CR 56:599
Unknown	D15S87	20	7	0.35	Endocrine	CR 56:599
Unknown	D15S97	32	8	0.25	Endocrine	CR 56:599
Unknown	GABRB3	31	7	0.23	Endocrine	CR 56:599
Unknown	D15S27	17	2	0.12	Esophageal	GCC 10:177
Unknown	D15S27	27	2	0.07	Esophageal	CR 54:2996
Unknown	D15S117	21	1	0.05	Head&Neck	CR 54:1152
Unknown	D15S118	17	1	0.06	Head&Neck	CR 54:4756
Unknown	D15S118	15	0	0	Head&Neck	CR 54:4756
Unknown	D15S118	6	3	0.5	Kidney	GCC 12:76
Unknown	D15S120-D15S127	21	1	0.05	Kidney	PNAS 92:2854
Unknown	D15S120-D15S127	6	0	0	Kidney	PNAS 92:2854
Unknown	D15S28	18	2	0.11	Kidney	CR 51:820
14-21	D15S1	10	1	0.1	Liver	JJCR 81:108
pter-q13	D15S24	26	3	0.12	Liver	CR 51:89
14-21	D15S1	4	0	0	Lung	NEJ 317:1109
14-21	D15S1	8	0	0	Lung	PN 84:9252
14-21	D15S1	5	2	0.4	Lung	NEJ 317:1109
14-21	D15S1	2	0	0	Lung	NEJ 317:1109
Unknown	D15S28	18	2	0.11	Lung	CR 52:2478
Unknown	D15S118	24	4	0.17	Melanoma	CR 56:589
14-21	D15S1	7	0	0	Neuroblastoma	CR 49:1095

Chromosome 15 - q Arm

11-12.0	D15S11	13	1	0.08	Ovary	IJC 54:546
Unknown	D15S2	11	4	0.36	Ovary	CR 53:2393
pter-q13	D15S24	31	2	0.06	Ovary	IJC 54:546
Unknown	D15S28	9	1	0.11	Ovary	CR 51:5118
26.1	FES	15	6	0.4	Ovary	BJC 69:429
pter-q13	D15S24	1	0	0	Pancreas	CR 51:2761
Unknown	D15S29-D15S1	9	0	0	Prostate	G 11:530
14-21	D15S1	9	4	0.44	Sarcoma	CR 52:2419
Unknown	D15S27	12	5	0.42	Sarcoma	CR 52:2419
14-21	D15S1	13	0	0	Stomach	CR 48:2980
Unknown	D15S86	32	5	0.16	Stomach	HG 92:244
pter-q13	D15S24	46	4	0.09	Testis	O 9:2245
Unknown	D15S86	21	2	0.1	Testis	GCC 13:249
Unknown	CYP19	27	0	0	Uterus	CR 54:4294
14-21	D15S1	6	1	0.17	Uterus	CR 51:5632
26.1	FES	36	5	0.14	Uterus	CR 54:4294
SUM		1015	173	0.17		

Chromosome 16 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Refe
13.3	HBZP1	6	0	0	Prostate	G-1
13.3	D16S85	7	0	0	Breast	CR
13.3	D16S85	62	5	0.08	Breast	GCC
13.3	D16S85	8	0	0	Liver	BJC
13.3	D16S85	11	0	0	Liver	BJC
13.3	D16S85	24	5	0.21	Ovary	CR
13.3	D16S85	11	1	0.09	Pancreas	BJC
13.3	D16S85	11	1	0.09	Stomach	HG
13.3	D16S85	22	3	0.14	Testis	GCC
13.3	D16S83	27	8	0.3	Breast	GCC
13.3	D16S83	31	6	0.19	Breast	CR
13.3	D16S83	16	2	0.12	Esophageal	CR
13.3	D16S83	11	0	0	Esophageal	CR
13.3	D16S83	19	5	0.26	Liver	CR
13.3	D16S83	16	1	0.06	Liver	CR
13.3	D16S83	15	6	0.4	Sarcoma	CR
13	D16S84	21	1	0.05	Breast	CR
13	D16S84	43	0	0	Breast	CR
pter-p13.3	D16S84	5	0	0	Cervix	GCC
pter-p13.3	D16S84	28	4	0.14	Esophageal	GCC
pter-p13.3	D16S84	14	1	0.07	Kidney	CR
pter-p13.3	D16S84	22	5	0.23	Lung	CR
pter-p13.3	D16S84	21	7	0.33	Ovary	CR
pter-p13.3	D16S84	9	2	0.22	Uterus	GCC
13.3	HBAI	22	5	0.23	Breast	CR
13.3	HBAI	47	1	0.02	Breast	CR
13.3	HBAI	22	5	0.23	Breast	CR
13.3	HBAI	11	9	0.82	Liver	CR
13.3	HBAI	36	16	0.44	Liver	PNA
Unknown	D16S414	10	0	0	Head&Neck	CR
Unknown	D16S414	19	3	0.16	Head&Neck	CR
Unknown	D16S414	6	3	0.5	Kidney	GCC
Unknown	D16S414	26	1	0.04	Melanoma	CR
13	D16S292	12	0	0	Ovary	BJC
pter-p13	D16S32	21	3	0.14	Breast	CR
pter-p13	D16S32	26	8	0.31	Liver	PNA
pter-p13	D16S32	16	4	0.25	Liver	BJC
pter-p13	D16S32	8	7	0.88	Liver	CR
13.1	MRP	13	5	0.38	Leukemia	LAN
13.11	D16S131	8	1	0.12	Breast	CR
13.11	D16S131	13	6	0.46	Liver	PNA
12.2	D16S159	34	6	0.18	Breast	CR
P11-P13	D16S159	29	1	0.03	Breast	CR
Unknown	D16S159	22	1	0.05	Liver	CR
Unknown	D16S159	22	1	0.05	Liver	CR
Unknown	Unknown	18	2	0.11	Brain	CR

Chromosome 16 - p Arm

12.2	D16S23	36	5	0.14	Breast	CR
13.2	D16S34	3	1	0.33	Breast	CR
13.2	D16S34	21	7	0.33	Breast	CR
PTER-P13	D16S35	26	4	0.15	Breast	CR
PTER-P13	D16S35	20	1	0.2	Cervix	CR
12-pter	Unknown	18	0	0	Colon	BJC
Unknown	D16S418	22	0	0	Endocrine	CR
Unknown	D16S404	20	2	0.1	Head&Neck	CR
Unknown	D16S404-D16S403-D16S414	22	0	0	Kidney	PNA
Unknown	D16S404-D16S403-D16S414	6	0	0	Kidney	PNA
13.2	D16S34	20	9	0.45	Liver	PNA
13.2	D16S34	8	5	0.62	Liver	CR
13.2	D16S34	6	3	0.5	Liver	CR
PTER-P13	D16S35	7	4	0.57	Liver	CR
PTER-P13	D16S35	24	9	0.38	Liver	PNA
pter-p13	D16S37	2	0	0	Liver	JJC
13.2	D16S34	27	4	0.15	Ovary	JJC
PTER-P13	D16S35	8	0	0	Prostate	PNA
PTER-P13	D16S35	8	0	0	Prostate	CSn
12-pter	Unknown	5	0	0	Stomach	BJC
PTER-P13	D16S35	25	5	0.2	Testis	O.J
Unknown	D16S291	18	1	0.06	Uterus	CR
SUM		1231	213	0.17		

Chromosome 16 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
16	D16S131	37	5	0.14	Breast	CR 54:5243
Unknown	D16S300	23	7	0.3	Breast	GCC 14:171
Unknown	D16S299	36	7	0.19	Breast	GCC 14:171
12.1	D16S304	24	12	0.5	Breast	GCC 14:171
22.1	TAT	43	16	0.37	Breast	CR 54:513
22.1	TAT	41	15	0.37	Breast	GCC 9:101
22.1	TAT	8	5	0.62	Liver	CR 52:1504
22.1	TAT	10	9	0.9	Liver	CR 54:281
22.1	TAT	23	13	0.57	Liver	PNAS 87:6791
22.1	TAT	25	13	0.52	Liver	PNAS 87:6791
22.1	TAT	29	14	0.48	Liver	PNAS 87:6791
Unknown	D16S408	20	3	0.15	Breast	JJCR 86:1054
13	CET	36	9	0.25	Breast	CR 54:513
21	CET	44	20	0.45	Liver	PNAS 87:6791
13-22.1	MT2	36	15	0.42	Liver	PNAS 87:6791
21	D16S151	43	16	0.37	Breast	CR 51:5794
21	D16S151	18	6	0.33	Breast	CR 54:513
21	D16S151	43	8	0.19	Esophageal	GCC 10:177
Unknown	D16S151	8	2	0.25	Liver	CR 51:89
21	D16S265	70	24	0.34	Breast	GCC 9:101
21	D16S265	58	19	0.33	Breast	BCRT 32:5
21	D16S265	19	3	0.16	Ovary	BJC 69:429
22.1	D16S38	35	14	0.4	Breast	CR 54:513
21-22.1	D16S186	28	15	0.54	Breast	GCC 14:171
21-22.1	D16S186	33	13	0.39	Breast	GCC 9:101
21-22.1	D16S186	27	6	0.22	Uterus	CR 54:4294
22.1	D16S318	33	13	0.39	Breast	GCC 9:101
22.1	D16S318	29	14	0.48	Breast	GCC 14:171
Unknown	D16S421	12	2	0.17	Breast	JJCR 86:1054
Unknown	D16S421	27	14	0.52	Breast	GCC 14:171
22.1	D16S4	28	16	0.57	Breast	CR 54:513
22.1	D16S4	29	14	0.48	Breast	GCC 9:101
22.1	D16S4	31	12	0.39	Liver	PNAS 87:6791
22.1	D16S4	9	5	0.56	Liver	CR 52:1504
22.1	D16S4	17	6	0.35	Ovary	CR 53:2393
22.1	D16S152	21	4	0.19	Breast	CR 54:513
22.1	HP	27	11	0.41	Breast	CR 54:513
22.1	HP	21	12	0.57	Breast	CR 51:5794
22.1	HP	29	15	0.52	Breast	GCC 9:101
22.1	HP	9	1	0.11	Cervix	CR 49:3598
22.1	HP	15	3	0.2	Colon	IJC 53:382
Unknown	HP	7	1	0.14	Liver	CR 51:89
Unknown	HP	10	4	0.4	Liver	CR 52:1504
22.1	HP	28	10	0.36	Liver	PNAS 87:6791
22.1	HP	14	8	0.57	Liver	JJCR 81:108
22.1	HP	13	7	0.54	Liver	JJCR 81:108

Chromosome 16 - q Arm

22.1	HP	20	5	0.25	Lung	PN 84:9252
22.1	HP	4	0	0	Neuroblastoma	CR 49:1095
Unknown	HP	24	2	0.08	Ovary	GO 42:137
22.1	HP	22	5	0.23	Ovary	IJC 54:546
22.1	HP	4	0	0	Prostate	G,CC 5:30
Unknown	HP	11	1	0.09	Stomach	CR 52:3099
22.1	HP	10	0	0	Stomach	CR 48:2908
22.1	HP	2	0	0	Testis	CCG 52:72
22.1	HP	2	0	0	Testis	CCG 52:72
22.1	HP	2	0	0	Testis	CCG 52:72
22.1	HP	4	0	0	Uterus	CR 51:5632
22.3-23.2	CTRB	34	9	0.26	Breast	CR 54:513
23.2	CTRB	4	2	0.5	Breast	CR 51:5794
23.2	CTRB	9	5	0.56	Liver	CR 52:1504
22.3-23.2	CTRB	38	17	0.45	Liver	PNAS 87:6791
23.3-24.1	D16S289	28	13	0.46	Breast	GCC 14:171
23.3-24.1	D16S289	57	21	0.37	Breast	GCC 9:101
23.3-24.1	D16S289	22	5	0.23	Uterus	CR 54:4294
24.2	D16S20	45	15	0.33	Breast	CR 54:513
22.1-24	D16S30	6	3	0.5	Breast	CR 54:513
Unknown	D16S511	32	15	0.47	Breast	GCC 14:171
Unknown	D16S402	12	5	0.42	Breast	JJCR 86:1054
Unknown	D16S402	38	20	0.53	Breast	GCC 14:171
Unknown	D16S402	13	2	0.15	Head&Neck	CR 54:1152
24.2-24.3	D16S157	21	9	0.43	Breast	CR 54:513
22-23	D16S157	9	4	0.44	Breast	CR 51:5794
24.2-24.3	D16S43	20	8	0.4	Breast	CR 54:513
Unknown	D16S155	11	2	0.18	Breast	CR 54:513
23-24	D16S156	61	30	0.49	Breast	CR 51:5794
24	APRT	33	17	0.52	Breast	CR 54:513
24	APRT	25	3	0.12	Breast	CR 53:3707
24	APRT	25	3	0.12	Breast	CR 53:4356
24	APRT	19	10	0.53	Breast	GCC 2:191
24	APRT	12	7	0.58	Breast	GCC 9:101
24	APRT	10	6	0.6	Liver	CR 52:1504
24	APRT	26	17	0.65	Liver	PNAS 87:6791
Unknown	D16S7	10	1	0.1	Brain	CR 49:6572
24	D16S7	21	3	0.14	Brain	CR 50:5784
24	D16S7	42	19	0.45	Breast	CR 50:7184
24	D16S7	8	6	0.75	Breast	CR 53:3804
24	D16S7	354	164	0.46	Breast	BJC 71:438
24	D16S7	59	30	0.51	Breast	GCC 9:101
24	D16S7	57	18	0.32	Breast	CR 53:4356
24	D16S7	57	18	0.32	Breast	CR 53:3707
24	D16S7	269	120	0.45	Breast	IC 74:2281
24.3	D16S7	68	32	0.47	Breast	CR 54:513

Chromosome 16 - q Arm

23-24	D16S7	138	59	0.43	Breast	CR 51:5794
Unknown	D16S7	83	23	0.28	Breast	JJCR 84:1159
Unknown	D16S7	35	1	0.03	Cervix	CR 54:4481
23-24	D16S7	7	2	0.29	Cervix	GCC 9:119
23-24	D16S7	32	6	0.19	Colon	IJC 53:382
23-24	D16S7	6	1	0.17	Esophageal	CR 51:2113
Unknown	D16S7	15	4	0.27	Esophageal	CR 54:2996
24	D16S7	29	3	0.1	Kidney	CR 51:820
Unknown	D16S7	33	12	0.36	Liver	CR 51:89
24	D16S7	53	24	0.45	Liver	PNAS 87:6791
23-24	D16S7	25	11	0.44	Liver	CR 54:281
24	D16S7	50	14	0.28	Liver	JJCR 84:893
24	D16S7	37	8	0.22	Lung	CR 57:2478
Unknown	D16S7	30	11	0.37	Ovary	CR 51:5118
24	D16S7	3	1	0.33	Pancreas	CR 54:2761
24	D16S7	15	4	0.27	Prostate	PNAS 87:8751
Unknown	D16S7	17	3	0.18	Prostate	BJU 73:390
24	D16S7	32	9	0.28	Sarcoma	CR 52:2419
24	D16S7	43	2	0.05	Testis	O 9:2245
Unknown	D16S7	16	0	0	Uterus	GCC 9:119
24.3	D16S413	41	21	0.51	Breast	GCC 14:171
24.3	D16S413	22	0	0	Endocrine	CR 56:599
24.3	D16S44	10	4	0.4	Breast	CR 54:513
24.3	D16S303	23	11	0.48	Breast	GCC 14:171
24.3	D16S303	42	18	0.43	Breast	GCC 9:101
13	MT2	29	9	0.31	Breast	CR 54:513
13	MT2	8	4	0.5	Liver	CR 52:1504
13	MT2	8	4	0.5	Liver	CR 52:1504
Unknown	D16S10	31	7	0.23	Breast	GCC 9:101
Unknown	D16S260	28	8	0.29	Breast	GCC 9:101
Unknown	D16S266	53	18	0.34	Breast	GCC 9:101
12.1	D16S27	26	7	0.27	Breast	CR 54:513
12.1	D16S27	27	9	0.33	Breast	GCC 9:101
Unknown	D16S301	38	16	0.42	Breast	GCC 9:101
Unknown	D16S305	58	20	0.34	Breast	GCC 9:101
Unknown	D16S320	65	20	0.31	Breast	GCC 9:101
Unknown	D16S398	56	16	0.29	Breast	GCC 9:101
Unknown	D16S5	29	11	0.38	Breast	GCC 9:101
22.1	E-cadherin	28	16	0.57	Breast	GCC 9:101
22.1	E-cadherin	41	27	0.66	Breast	EMBO 14:6107
Unknown	D16S422	21	4	0.19	Head&Neck	CR 54:4756
Unknown	D16S422	20	0	0	Head&Neck	CR 54:4756
Unknown	SPN	22	3	0.14	Head&Neck	CR 54:1152
Unknown	D16S413-D16S402	21	0	0	Kidney	PNAS 92:2854
Unknown	D16S413-D16S402	6	0	0	Kidney	PNAS 92:2854
Unknown	D16S:422-419	6	3	0.5	Kidney	GCC 12:76

Chromosome 16 - q Arm

Unknown	Unknown	3	0	0	Liver	BJC 67:1007
Unknown	Unknown	6	0	0	Liver	BJC 64:1083
Unknown	DIGS:422-419	21	0	0	Melanoma	GR 56:589
Unknown	Unknown	16	5	0.31	Prostate	CSurveys 11:
SUM		4382	1588	0.36		

Chromosome 17 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D17S34	35	5	0.14	Brain	AJP 145:11
13.3	D17S34	82	29	0.35	Breast	AJP 140:21
13.3	D17S34	77	52	0.68	Breast	CR 54:4200
13-TER	D17S34	72	30	0.42	Breast	CGC 76:106
Unknown	D17S34	70	41	0.59	Breast	O 9:781
13.3	D17S34	44	33	0.75	Breast	GCC 4:113
13.3	D17S34	36	22	0.61	Breast	CR 53:1637
Unknown	D17S34	11	6	0.55	Cervix	CGC 79:74
13.3	D17S34	68	34	0.5	Colon	BJC 30A:66
13.3	D17S34	6	5	0.83	Colon	Science Ap 1989:217
13.3	D17S34	6	3	0.5	Head&Neck	AJP 142:11
Unknown	D17S34	12	1	0.08	Head&Neck	CR 52:4787
13.3	D17S34	20	2	0.1	Liver	O 9:493
13.3	D17S34	10	8	0.8	Liver	BJC 64:108
13.3	D17S34	9	4	0.44	Liver	BJC 67:100
13.3	D17S34	23	12	0.52	Ovary	IJC 54:85
13.3	D17S34	20	18	0.9	Ovary	IJC 54:220
Unknown	D17S34	43	18	0.42	Ovary	CR 56:606
13.3	D17S34	11	0	0	Pancreas	CR 54:2761
13.3	D17S34	17	3	0.18	Prostate	CSurveys 1
13.3	D17S34	18	3	0.17	Prostate	PNAS 87:87
13.3	D17S34	7	5	0.71	Sarcoma	CR 53:468
13.3	D17S34	9	0	0	Sarcoma	CR 53:468
13.3	D17S34	10	4	0.4	Sarcoma	CR 53:468
13.3	D17S34	4	2	0.5	Sarcoma	CR 53:468
13.3	D17S34	20	0	0	Testis	GCC 13:249
13.3	D17S849	26	16	0.62	Breast	HMG 4:2047
13.3	D17S926	12	7	0.58	Breast	HMG 4:2047
13.3	D17S30	54	20	0.37	Breast	CR 53:1637
13.3	D17S30	98	57	0.58	Breast	Lan 336:76
13.3	D17S30	59	30	0.51	Breast	JNCI 84:50
13.3	D17S30	52	27	0.52	Breast	PNAS 88:38
13.3	D17S30	51	8	0.16	Breast	HG 91:6
13.3	D17S30	34	16	0.47	Breast	CR 50:7184
13.3	D17S30	33	17	0.52	Breast	ANYAS p.13
13.3	D17S30	3	0	0	Breast	CR 53:2947
13.3	D17S30	6	3	0.5	Cervix	GCC 5:119
13.3	D17S30	39	27	0.69	Colon	CR 50:7166
13.3	D17S30	60	38	0.63	Colon	BJC 30A:66
13.3	D17S30	65	40	0.62	Esophageal	GCC 10:177
13.3	D17S30	51	36	0.71	Head&Neck	O 10:1217
13.3	D17S30	5	2	0.4	Liver	BJC 67:100
13.3	D17S30	26	14	0.54	Liver	CR 54:89
13.3	D17S30	37	23	0.62	Lung	CR 52:2478
13.3	D17S30	16	4	0.25	Melanoma	GCC 7:169

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13.3	D17S30	14	9	0.64	Ovary	CR 50:2724
13.3	D17S30	21	18	0.86	Ovary	IJC 54:85
13.3	D17S30	46	37	0.8	Ovary	CR 56:606
13.3	D17S30	41	27	0.66	Ovary	CR 51:5932
13.3	D17S30	7	0	0	Prostate	GCC 11:119
13.3	D17S30	3	0	0	Sarcoma	CR 53:468
13.3	D17S30	6	4	0.67	Sarcoma	CR 53:468
13.3	D17S30	3	0	0	Sarcoma	CR 53:468
13.3	D17S30	6	0	0	Sarcoma	CR 53:468
13.3	D17S30	17	16	0.94	Sarcoma	CR 49:6247
13.3	D17S30	15	3	0.2	Uterus	GCC 9:119
13.3	D17S28	11	4	0.36	Brain	CR 49:6572
13.3	D17S28	22	3	0.14	Brain	AJP 145:11
13.3	D17S28	12	4	0.33	Brain	CR 49:6572
13.3	D17S28	27	11	0.41	Breast	CR 54:6270
13.3	D17S28	62	15	0.24	Breast	CGC 46:406
13.3	D17S28	37	26	0.7	Breast	CR 54:4200
13.3	D17S28	11	4	0.36	Breast	HMG 4:2047
13.3	D17S28	23	12	0.52	Breast	CR 53:1637
13.3	D17S28	27	4	0.15	Cervix	CR 54:4481
13.3	D17S28	14	1	0.07	Cervix	BJC 67:71
13.3	D17S28	7	5	0.71	Colon	Science,Ro 1989:217
13.3	D17S28	13	8	0.62	Colon	GCC 3:468
13.3	D17S28	12	4	0.33	Colon	GCC 48:167
13.3	D17S28	2	0	0	Head&Neck	CR 52:4787
13.3	D17S28	11	0	0	Kidney	JU 150:329
13.3	D17S28	3	1	0.33	Liver	CR 53:368
13.3	D17S28	3	3	1	Lung	CR 49:5130
13.3	D17S28	16	2	0.12	Ovary	IJC 52:575
13.3	D17S28	8	6	0.75	Ovary	CR 50:2724
13.3	D17S28	23	15	0.65	Ovary	CR 56:606
13.3	D17S28	6	4	0.67	Ovary	IJC 54:85
13.3	D17S28	18	14	0.78	Ovary	IJC 54:220
13.3	D17S28	3	1	0.33	Pancreas	CR 54:2761
13.3	D17S28	3	0	0	Pancreas	GCC 3:468
13.3	D17S28	10	2	0.2	Stomach	BJC 53:750
13.3	D17S28	7	0	0	Stomach	HG 89:445
13.3	D17S28	29	12	0.41	Testis	CR 9:2245
13.3	D17S28	1	1	1	Uterus	CR 51:5632
Unknown	Unknown	20	10	0.5	Bladder	JU 153:109
Unknown	Unknown	76	21	0.28	Brain	CR 56:164
13.3	D17S34-S5	13	7	0.54	Brain	CR 54:1397
13.3	D17S34-S5	20	11	0.55	Brain	CR 54:1397
13.3	D17S5	22	4	0.18	Brain	AJP 145:11
13.3	D17S5	16	6	0.38	Brain	IJC 63:372
13.3	D17S5	13	6	0.46	Brain	CR 49:6572

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13.3	D17S5	11	6	0.55	Brain	CR 49:6572
13.3	Unknown	74	20	0.27	Breast	AJP 140:721
13.3	D17S5	62	26	0.42	Breast	JJCR 84:11
13.3	D17S5	68	37	0.54	Breast	CR 51:3807
13.3	D17S5	57	28	0.49	Breast	BCRT 28:23
13.3	D17S5	4	2	0.5	Breast	CR 53:3804
13.3	D17S5	29	16	0.55	Breast	GCC 2:191
13.3	D17S5	50	8	0.16	Breast	CR 50:4356
13.3	D17S5	465	224	0.48	Breast	BJC 71:438
13.3	D17S5	34	15	0.44	Breast	BMC 4:2047
13.3	D17S5	82	53	0.65	Breast	CR 54:4200
13.3	D17S5	75	21	0.28	Breast	CGC 16:105
13.3	D17S5	354	174	0.49	Breast	C 74:2281
13.3	D17S5	99	18	0.46	Breast	IJC 55:197
13.3	D17S5	42	25	0.6	Breast	IJC 50:528
13.3	D17S5	40	22	0.55	Breast	GCC 4:251
13.3	D17S5	125	63	0.5	Breast	CR 51:5794
13.3	D17S5	61	26	0.43	Breast	BC 90:635
13.3	D17S5	52	27	0.52	Breast	PNAS 88:38
13.3	D17S5	15	4	0.27	Cervix	CGC 19:74
13.3	D17S5	12	1	0.08	Cervix	BJC 67:71
13.3	D17S5	32	5	0.16	Cervix	CR 54:4401
13.3	Unknown	7	6	0.86	Colon	Science Ap 1989:217
13.3	D17S5	35	24	0.69	Colon	BJC 69:750
13.3	D17S5	19	7	0.37	Colon	CCG 48:167
13.3	D17S5	5	3	0.6	Colon	O 8:1991
13.3	D17S5	27	21	0.78	Colon	IJC 53:382
13.3	D17S5	17	7	0.41	Colon	GCC 3:465
13.3	D17S5	26	10	0.38	Colon	S 241:961
13.3	D17S34-S5	24	11	0.46	Esophageal	CR 52:6525
13.3	D17S5	22	10	0.45	Esophageal	CR 51:2113
13.3	Unknown	6	5	0.83	Head&Neck	AJP 142:11
13.3	D17S5	11	2	0.18	Head&Neck	CR 52:1494
13.3	D17S5	48	8	0.17	Kidney	CR 51:5817
13.3	D17S5	23	6	0.26	Kidney	JU 150:129
13.3	D17S5	15	5	0.33	Kidney	CR 51:820
13.3	D17S5	31	5	0.16	Kidney	CR 51:1544
13.3	D17S5	15	1	0.07	Kidney	CR 51:1071
13.3	D17S5	2	1	0.5	Kidney	CR 51:1544
13.3	D17S5	20	3	0.15	Liver	O 8:491
13.3	D17S5	14	3	0.21	Liver	CR 51:4367
13.3	D17S5	31	15	0.48	Liver	CR 53:368
13.3	D17S5	9	3	0.33	Liver	BJC 64:108
13.3	D17S34-S5	11	11	1	Lung	CR 49:5130
13.3	D17S5	6	6	1	Lung	CR 55:28
13.3	D17S34-S5	38	25	0.66	Ovary	O 7:2069

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13.3	D17S34-S5	6	2	0.33	Ovary	O 7:2069
13.3	D17S5	37	28	0.76	Ovary	IJC 54:220
13.3	D17S5	28	12	0.43	Ovary	CR 51:5118
13.3	D17S5	33	8	0.27	Ovary	IJC 54:916
13.3	D17S5	34	7	0.21	Ovary	IJC 52:575
13.3	D17S5	41	27	0.66	Ovary	O 7:1059
13.3	D17S5	28	15	0.54	Ovary	GO 47:137
13.3	D17S1	5	0	0	Pancreas	GCC 3:466
13.3	D17S5	8	0	0	Pancreas	BJC 65:809
13.3	D17S5	54	2	0.5	Pancreas	CR 51:2761
13.3	D17S5	27	1	0.04	Pediatric	CR 50:3279
13.3	D17S5	8	6	0.75	Sarcoma	GCC 53:45
13.3	D17S5	22	16	0.73	Sarcoma	CR 52:2419
13.3	D17S5	60	38	0.63	Stomach	CR 52:2419
13.3	D17S5	38	19	0.5	Stomach	CR 51:2926
13.3	D17S5	40	2	0.04	Stomach	GCC 3:466
13.3	D17S5	24	9	0.38	Stomach	HG 92:244
13.3	D17S5	30	6	0.2	Testis	O 9:2245
13.3	D17S5	9	4	0.44	Uterus	CR 51:5632
13.3	D17S379	22	15	0.68	Ovary	CR 56:606
13.3	ABR	29	6	0.21	Ovary	CR 56:606
Unknown	D17S65	16	10	0.62	Breast	CR 54:4200
13	D17S65	16	11	0.69	Breast	GE 5:554
13	D17S65	7	7	1	Colon	S:April 16
13	D17S1	15	3	0.2	Brain	AJP 145:11
13	D17S1	15	2	0.13	Brain	AJP 145:11
13	D17S1	21	4	0.19	Breast	HG 91:6
13	D17S1	20	9	0.45	Breast	GCC 2:191
13	D17S1	29	9	0.31	Breast	CR 53:4356
13	D17S1	7	2	0.29	Cervix	CR 49:3598
13	D17S1	14	6	0.43	Colon	CR 50:7166
13	D17S1	9	0	0	Colon	N 331:274
13	D17S1	2	2	1	Colon	S:April 16
13	D17S1	12	4	0.33	Colon	S 241:961
13	D17S1	30	13	0.43	Head&Neck	O 10:1217
13	D17S1	7	1	0.14	Liver	JJCR 84:10
13	D17S1	11	2	0.18	Liver	CR 53:368
13	D17S1	3	1	0.33	Lung	PNAS 86:50
13	D17S1	9	8	0.89	Lung	PNAS 86:50
13	D17S1	17	8	0.47	Lung	PN 81:9252
13	D17S1	7	7	1	Lung	CR 49:5130
13	D17S1	11	2	0.18	Lung	PNAS 86:50
13	D17S1	4	0	0	Neuroblastoma	CR 49:1095
13	D17S1	5	0	0	Sarcoma	CR 53:468
13	D17S1	3	1	0.33	Sarcoma	CR 53:468
13	D17S1	3	0	0	Sarcoma	CR 53:468

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13	D17S1	8	7	0.88	Sarcoma	CR 52:2419
13	D17S1	2	0	0	Sarcoma	CR 52:2419
13	D17S1	13	12	0.92	Sarcoma	CR 49:6247
13	D17S1	15	14	0.93	Stomach	CR 49:3899
13	D17S1	10	0	0	Stomach	CR 48:2988
13	D17S1	6	4	0.67	Uterus	CR 52:5972
Unknown	D17S796	17	0	0	Endocrine	CR 56:599
Unknown	D17S796	11	11	0.36	Head&Neck	CR 54:4756
Unknown	D17S796	33	0	0	Head&Neck	CR 54:4756
Unknown	D17S796	6	3	0.5	Kidney	CR 52:716
Unknown	D17S796	32	5	0.16	Melanoma	CR 56:589
12.0-13	D17S906	19	3	0.16	Prostate	GCC 49:278
13.1	D17S31	9	2	0.22	Brain	CR 49:6572
13.1	D17S31	3	1	0.33	Brain	AJP 145:11
13.1	D17S31	8	4	0.5	Brain	CR 49:6572
13.1	D17S31	21	7	0.33	Breast	BJC 63:63
13.1	D17S31	54	24	0.44	Breast	Lan 336:76
13.1	D17S31	34	22	0.65	Breast	CR 51:6200
13.1	D17S31	87	37	0.43	Breast	CR 51:5794
13.1-11.2	D17S31	25	11	0.44	Breast	IJC 50:528
13.1	D17S31	2	1	0.5	Breast	CR 53:2947
13.1	D17S31	11	1	0.09	Cervix	BJC 62:71
13.1-11.2	D17S31	16	7	0.44	Colon	CR 50:7166
13.1	D17S31	6	6	1	Colon	CR 51:816
13.1	D17S31	15	9	0.6	Esophageal	CR 54:2996
13.1	D17S31	29	18	0.62	Head&Neck	O 10:1215
13.1-11.2	D17S31	28	5	0.18	Kidney	CR 51:5817
13.1	D17S31	25	0	0	Kidney	JNCI 50:129
13.1-11.2	D17S31	16	6	0.38	Liver	CR 51:89
13.1	D17S31	21	12	0.57	Liver	CR 53:368
13.1	D17S31	17	7	0.41	Ovary	IJC 54:546
13.1	D17S31	7	2	0.29	Ovary	IJC 54:65
13.1	D17S31	11	8	0.73	Ovary	IJC 54:220
13.1	D17S31	7	4	0.57	Ovary	BJC 65:40
13.1	D17S31	6	2	0.33	Ovary	CR 56:606
13.1	D17S31	3	1	0.33	Pancreas	CR 54:2761
13.1-11.2	D17S31	17	12	0.71	Sarcoma	CR 52:2419
13.1	D17S31	15	15	1	Sarcoma	CR 49:6247
13.1	D17S31	12	9	0.75	Sarcoma	CR 52:2419
13.1	TP53	7	0	0	Bladder	HG 91:455
13.1	TP53	21	9	0.43	Brain	CR 54:1397
Unknown	TP53	1	0	0	Brain	AJP 145:11
13.1	TP53	45	6	0.13	Brain	O 6:1313
13.1	TP53	6	2	0.33	Brain	CR 49:6572
13.1	TP53	22	9	0.41	Brain	CGC 74:139
13.1	TP53	38	10	0.29	Brain	CR 52:1427

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13.1	TP53	15	7	0.47	Brain	CR 54:1397
13.1	TP53	6	2	0.33	Brain	CR 49:6572
13.1	TP53	31	22	0.71	Breast	BJC 68:64
Unknown	TP53	63	17	0.27	Breast	BCRT 28:23
13.1	TP53	61	14	0.23	Breast	CGC 76:106
Unknown	TP53	19	6	0.32	Breast	CR 51:6194
13.1	TP53	44	28	0.64	Breast	HG 90:635
13.1	TP53	35	13	0.37	Breast	IJC 50:520
13.1	TP53	70	26	0.37	Breast	CR 51:5794
13.1	TP53	65	13	0.2	Breast	BJCR 6:311
Unknown	TP53	11	6	0.55	Breast	CR 52:2624
13.1	TP53	81	22	0.27	Breast	Ban 336:76
13.1	TP53	25	10	0.4	Breast	GCC 4:113
13.1	TP53	36	10	0.28	Breast	BJC 69:744
13.1	TP53	12	5	0.42	Breast	CR 53:2947
13.1	TP53	110	72	0.65	Breast	CR 54:4200
13.1	TP53	36	15	0.42	Breast	CR 53:1637
13.1	TP53	17	9	0.53	Breast	GCC 4:103
13.1	TP53	41	34	0.83	Breast	IJC 57:498
Unknown	TP53	16	0	0	Cervix	GGC 19:74
13.1	TP53	9	1	0.11	Cervix	BJC 67:71
Unknown	TP53	6	3	0.5	Cervix	GCC 4:119
13.1	TP53	21	5	0.24	Cervix	CR 54:4481
13.1	TP53	17	8	0.47	Colon	CR 52:1741
13.1	TP53	6	5	0.83	Colon	GAST 107:3
Unknown	TP53	23	15	0.65	Colon	BJC 30A:26
Unknown	TP53	48	38	0.79	Colon	O 8:1391
Unknown	TP53	26	22	0.85	Colon	GAS 103:16
13.1	TP53	30	17	0.57	Colon	GAST 104:1
Unknown	TP53	6	4	0.67	Colon	O 9:991
13.1	TP53	25	12	0.48	Colon	HP 25:1069
13.1	TP53	14	8	0.57	Colon	CR 50:7166
13.1	TP53	17	8	0.47	Colon	JNCI 84:11
13.1	TP53	17	7	0.41	Colon	JNCI 84:11
13.1	TP53	17	10	0.59	Colon	IJC 53:382
13.1	TP53	25	14	0.56	Colon	CR 52:3965
13.1	TP53	12	10	0.83	Colon	CR 51:4436
13.1	TP53	27	15	0.56	Esophageal	C 73:2472
13.1	TP53	14	10	0.71	Esophageal	C 71:1933
Unknown	TP53	47	27	0.57	Esophageal	CR 52:6525
13.1	TP53	14	7	0.5	Head&Neck	CR 54:1152
Unknown	TP53	22	14	0.64	Head&Neck	O 9:2077
13.1	TP53	27	15	0.56	Head&Neck	C 73:2472
13.1	TP53	39	21	0.54	Head&Neck	O 10:1217
13.1	TP53	20	4	0.2	Kidney	CR 51:5817
Unknown	TP53	40	5	0.12	Kidney	BJC 69:230

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13.1	TP53	2	0	0	Kidney	GCC 12:76
13.1	TP53	10	6	0.6	Kidney	IJC 64:899
13.1	TP53	16	3	0.19	Kidney	CR 51:820
Unknown	TP53	65	9	0.14	Leukemia	LB 86:4587
13.1	TP53	50	14	0.28	Liver	JJCR 84:89
13.1	TP53	13	6	0.86	Liver	CR 53:6520
Unknown	TP53	4	1	0.25	Liver	CARC 17:14
13.1	TP53	64	37	0.58	Liver	C 73:1
Unknown	TP53	19	11	0.58	Liver	CR 54:281
13.1	TP53	5	1	0.2	Liver	O 8:2503
13.1	TP53	7	3	0.43	Liver	CR 51:89
13.1	TP53	24	10	0.71	Lung	CR 54:5643
13.1	TP53	57	21	0.37	Lung	O 10:937
13.1	TP53	7	5	0.71	Lung	CR 54:5643
13.1	TP53	3	2	0.67	Lung	CR 54:5643
13.1	TP53	3	0	0	Melanoma	GCC 2:169
Unknown	TP53	28	7	0.25	Melanoma	BJC 69:253
13.1	TP53	42	19	0.45	Ovary	CR 56:606
13.1	TP53	12	5	0.42	Ovary	IJC 54:546
13.1	TP53	18	10	0.56	Ovary	BJC 63:40
13.1	TP53	9	6	0.67	Ovary	IJC 54:85
13.1	TP53	9	2	0.22	Ovary	IJC 52:515
13.1	TP53	23	18	0.78	Ovary	IJC 54:220
13.1	TP53	18	12	0.67	Ovary	BJC 69:429
13.1	TP53	12	3	0.25	Ovary	CR 51:5118
13.1	TP53	20	16	0.8	Ovary	CR 51:5171
Unknown	TP53	35	26	0.74	Ovary	BJC 72:883
13.1	TP53	7	1	0.14	Ovary	O 7:2069
13.1	TP53	2	1	0.5	Ovary	O 7:2069
13.1	TP53	32	18	0.56	Ovary	O 7:2069
13.1	TP53	13	3	0.23	Ovary	O 7:2069
13.1	TP53	7	5	0.71	Pancreas	GCC 15:157
13.1	TP53	27	3	0.11	Prostate	AJP 145:28
13.1	TP53	8	3	0.38	Prostate	JU 151:107
13.1	TP53	4	0	0	Prostate	AJP 147:11
Unknown	TP53	5	3	0.6	Sarcoma	CR 53:468
Unknown	TP53	4	1	0.25	Sarcoma	CR 53:468
Unknown	TP53	7	1	0.14	Sarcoma	CR 53:468
Unknown	TP53	12	6	0.5	Sarcoma	CR 53:468
Unknown	TP53	63	23	0.37	Stomach	JJ 72:232
13.1	TP53	16	5	0.31	Stomach	CGC 75:45
Unknown	TP53	5	1	0.2	Testis	GCC 6:92
13.1	TP53	7	3	0.43	Testis	O 9:2245
13.1	TP53	9	2	0.22	Uterus	GCC 9:149
13.1	TP53	3	1	0.33	Uterus	CR 51:5632
13.1	TP53	4	1	0.25	Uterus	CR 51:5632

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Unknown	TP53	28	3	0.11	Uterus	CR 54:4294
13.1	D17S786	27	4	0.15	Cervix	CR 56:597
13.1	D17S786	2	0	0	Kidney	GCC 12:76
12	D17S520	14	7	0.5	Brain	CR 54:1997
12	D17S520	20	13	0.65	Brain	CR 54:1397
13.1	D17S520	31	15	0.48	Head&Neck	O 9:2077
12	D17S520	19	11	0.58	Ovary	BJC 69:429
13.1	D17S520	26	2	0.08	Uterus	CR 54:4294
13.1	MYH2	10	5	0.5	Brain	CR 49:6572
13.1	MYH2	8	2	0.25	Brain	CR 49:6572
13.1	MYH2	14	1	0.07	Brain	AJP 145:11
13.1	MYH2	14	10	0.71	Colon	IJC 53:382
13.1	MYH2	5	2	0.4	Liver	CR 53:368
13.1	MYH2	10	2	0.2	Liver	CR 49:6572
13.1	MYH2	10	10	1	Lung	CR 49:5130
13.1	MYH2	14	3	0.21	Ovary	IJC 54:846
13.1	MYH2	15	12	0.8	Sarcoma	CR 49:6247
13.1	MYH2	12	6	0.5	Sarcoma	CR/52:2419
13.1	MYH2	19	8	0.42	Stomach	CR 52:3099
13.1	MYH2	20	6	0.3	Uterus	CR 51:5632
12	D17S67	8	4	0.5	Brain	AJP 145:11
12	D17S67	35	22	0.63	Breast	CR 54:4200
12	D17S67	12	11	0.92	Breast	GE 5:554
12	D17S67	1	1	1	Colon	Science Ap 1989:217
12	D17S67	22	10	0.45	Ovary	IJC 54:546
12	D17S67	16	7	0.44	Ovary	CR 56:606
13.1	EW505	3	2	0.67	Colon	Science Ap 1989:217
13.1	UC 10-41	4	3	0.75	Colon	Science Ap 1989:217
13.1	EW401	3	1	0.33	Colon	Science Ap 1989:217
13.1	EW402	2	1	0.5	Colon	Science Ap 1989:217
13.1	EW405	3	1	0.33	Colon	Science Ap 1989:217
13.1	D17S29	15	1	0.07	Brain	CR 49:6572
13.1	D17S29	9	1	0.11	Brain	CR 49:6572
13.1	D17S29	2	0	0	Colon	Science Ap 1989:217
13.1	CHRNB1	26	14	0.54	Head&Neck	O 9:2077
13.1	CHRNB1	22	8	0.36	Head&Neck	CR 54:1152
13.1	CHRNB1	28	14	0.5	Ovary	CR 56:606
11.2-12	D17S261	6	2	0.33	Brain	CR 54:1397
11.2-12	D17S261	7	3	0.43	Brain	CR 54:1397
11.2-12	D17S261	19	8	0.42	Leukemia	BJH 3:449
12-11.2	D17S71	15	2	0.13	Brain	AJP 145:11

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12-11.2	D17S71	3	2	0.67	Breast	GE 5:554
12-11.2	D17S71	18	15	0.83	Colon	IJC 53:382
12-11.2	D17S71	9	3	0.33	Liver	CR 56:368
12-11.2	D17S71	10	10	1	Lung	CR 49:5130
12-11.2	D17S71	11	7	0.64	Ovary	GO 47:137
12-11.2	D17S71	20	11	0.55	Ovary	GO 47:137
12-11.2	D17S71	12	6	0.5	Sarcoma	CR 52:6247
12-11.2	D17S71	9	5	0.56	Sarcoma	CR 52:2419
12-11.2	D17S71	16	5	0.31	Uterus	CR 56:5622
13.1	D17S122	23	4	0.17	Brain	AJP 145:11
13.1	D17S122	29	11	0.38	Head&Neck	O 8:1053
13.1	D17S122	12	7	0.58	Head&Neck	CR 54:1152
Unknown	D17S58	17	2	0.12	Brain	AJP 145:11
11.2-11.1	D17S58	21	7	0.33	Breast	GE 5:554
11.2-11.1	D17S58	63	35	0.56	Breast	CR 54:4200
Unknown	D17S58	35	14	0.4	Breast	O 8:781
11.2-11.1	D17S58	10	10	1	Cervix	BJC 67:71
11.2-11.1	D17S58	5	1	0.2	Colon	Science Ap 1989:217
Unknown	D17S58	9	0	0	Head&Neck	CR 52:1787
11.2-11.1	D17S58	11	9	0.82	Ovary	IJC 54:85
Unknown	D17S58	19	12	0.63	Ovary	CR 56:696
Unknown	D17Z1	27	1	0.04	Breast	GE 5:554
Unknown	D17Z1	27	1	0.04	Breast	GE 5:554
D17S5-D17S58	Unknown	21	8	0.38	Bladder	CR 51:5405
Unknown	CHRNB2-TP53	30	18	0.6	Bladder	CR 55:5213
Unknown	Unknown	32	13	0.41	Brain	CR 50:5784
12-11.2	D17S121	17	3	0.18	Brain	AJP 145:11
Unknown	D17S5:28-31	14	0	0	Brain	CGC 73:122
Unknown	D17S5:28-31	25	6	0.24	Brain	CGC 73:122
Unknown	D17S5:28-31	15	5	0.33	Brain	CGC 73:122
Unknown	D17S66	15	2	0.13	Brain	AJP 145:11
13.3	Unknown	28	10	0.36	Breast	HMG 4:2047
13	Unknown	51	17	0.33	Breast	Lan 336:76
13.3	Unknown	27	16	0.59	Breast	HMG 4:2047
13.3	Unknown	22	9	0.41	Breast	HMG 4:2047
13.1-13.3	Unknown	88	38	0.43	Breast	CR 51:5794
13.1	Unknown	16	6	0.38	Bladder	CR 53:2617
13.3	Unknown	21	7	0.33	Breast	HMG 4:2047
13.3	D17S1174	7	3	0.43	Breast	HMG 4:2047
13	D17S513	17	6	0.35	Breast	CR 53:2947
Unknown	D17S66	7	7	1	Breast	CR 54:4200
13	Unknown	15	0	0	Cervix	BJC 67:71
13.3	Unknown	1	1	1	Colon	S:April 16
13.3	Unknown	3	3	1	Colon	S:April 16
13.3	Unknown	1	1	1	Colon	S:April 16
13.3	Unknown	4	4	1	Colon	S:April 16

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13.1	Unknown	2	2	1	Colon	ScienceAp 1989:217
Unknown	HF-12	12	6	0.5	Colon	JNCI 84:11
13	D17S513	32	20	0.62	Esophageal	C 73:2472
13	D17S513	32	20	0.62	Head&Neck	C 73:2472
13.3	D17S786	31	6	0.19	Head&Neck	[illegible]
13.2	CI17-732	35	1	0.03	Kidney	BJC 69:230
Unknown	D17S849-D17S796	6	0	0	Kidney	PNAS 92:28
Unknown	D17S849-D17S796	21	1	0.05	Kidney	PNAS 92:28
Unknown	D17S786-799	23	4	0.17	Leukemia	CR 55:5377
Unknown	Unknown	30	28	0.93	Lung	CR 54:2322
13	Unknown	19	10	0.53	Ovary	BJC 65:40
Unknown	D17S1-D17S28	15	2	0.13	Ovary	IJC 54:546
13.2	D17S260	21	10	0.48	Ovary	CR 56:606
13.1-13.3	D17S34-D17S28-D17S5-D17S379-P53-D17S513	7	7	1	Ovary	AJHG 55:66
13.1-13.3	D17S34-D17S28-D17S5-D17S379-P53-D17S513	2	2	1	Ovary	AJHG 55:66
13.1-13.3	D17S34-D17S28-D17S5-D17S379-P53-D17S513	12	12	1	Ovary	AJHG 55:66
13.1-13.3	D17S34-D17S28-D17S5-D17S379-P53-D17S513	1	1	1	Ovary	AJHG 55:66
Unknown	D17S5-34-71-MYH2	36	29	0.81	Ovary	CR 53:2393
13	D17S513	36	16	0.44	Ovary	CR 56:606
13.3	D17S578	29	12	0.41	Ovary	CR 56:606
13.3	D17S654	27	17	0.63	Ovary	CR 56:606
13.3	D17S695	41	18	0.44	Ovary	CR 56:606
Unknown	D17S:34-5-28-31	19	12	0.63	Ovary	CGC 85:43
Unknown	TP53-D17S:515-520-513	18	9	0.5	Ovary	BJC 72:133
Unknown	D17S1-D17S28	7	0	0	Prostate	G 11:530
12.0-13	D17S1149	15	4	0.27	Prostate	GCC 13:278
Unknown	D17S1-D17S28	8	2	0.25	Stomach	GCC 3:468
Unknown	Unknown	19	2	0.11	Testis	G 5:134
Unknown	D17S134	17	0	0	Testis	GCC 13:249
Unknown	D17S30-D17S787	24	2	0.08	Testis	LI 73:606
Unknown	12G6	22	2	0.09	Uterus	CR 54:4294
SUM		10343	4539	0.44		

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Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	D17S146	6	4	0.67	Ovary	IJC 54:220
11.2-12	D17S33	8	1	0.12	Brain	CR 49:6572
11.2-12	D17S33	9	0	0	Brain	CR 49:6572
11.2-12	D17S33	59	13	0.22	Breast	CR 51:5794
11.2-12	D17S33	7	1	0.14	Ovary	CR 51:16
11.2-12	D17S33	7	2	0.29	Sarcoma	CR 52:2419
11.2-12	D17S33	9	2	0.22	Sarcoma	CR 49:6267
11.2-12	CRYB1	13	0	0	Brain	AJP 145:1175
11.2-12	CRYB1	28	2	0.07	Breast	GCC 4:313
11.2-12	CRYB1	16	0	0	Colon	JNCI 84:1100
Unknown	D17S117	15	6	0.4	Breast	CR 53:5617
Unknown	D17S73	25	6	0.24	Breast	O 8:781
CEN-12	D17S73	27	26	0.97	Breast	CR 53:5617
CEN-12	D17S73	7	3	0.43	Ovary	IJC 54:85
11.2-12	D17S907	18	4	0.22	Prostate	GCC 13:278
11.2-12	THRA1	37	10	0.27	Breast	CR 54:2549
11.2-12	THRA1	66	17	0.26	Breast	GCC 12:58
11.2-12	THRA1	14	11	0.79	Breast	CR 52:2624
11.2-12	THRA1	17	7	0.41	Breast	AJOG 172:908
11.2-12	THRA1	13	5	0.38	Esophageal	CL 97:129
11.2-12	THRA1	17	12	0.71	Ovary	AJOG 172:908
11.2-12	THRA1	20	1	0.05	Ovary	IJC 54:220
13.1	TCF2	26	7	0.27	Head&Neck	ONS-2077
21.1	RARA	11	6	0.55	Ovary	IJC 54:85
11.2-12	D17S250	1	0	0	Bladder	HG 94:231
21	D17S250	5	1	0.2	Breast	CR 54:6069
21	D17S250	81	17	0.21	Breast	CR 54:2549
21	D17S250	78	18	0.23	Breast	GCC 11:58
11.2-12	D17S250	26	5	0.19	Breast	O 8:781
11.2-12	D17S250	6	1	0.17	Breast	HG 94:231
11.2-12	D17S250	14	7	0.5	Breast	CR 52:2624
21	D17S250	11	2	0.18	Esophageal	CL 97:129
11.2-12	D17S250	19	5	0.26	Head&Neck	CR 54:1152
11.2-12	D17S250	2	0	0	Ovary	HG 94:231
11.2-12	D17S250	22	14	0.64	Ovary	BJC 69:629
11.2-12	D17S250	20	2	0.1	Prostate	O 11:1241
21	D17S250	20	2	0.1	Prostate	CR 55:1002
21	PHB	4	3	0.75	Ovary	IJC 54:85
Unknown	PHB	9	9	1	Ovary	IJC 54:220
21	D17S800	1	0	0	Bladder	HG 94:231
21	D17S800	7	6	0.86	Breast	CR 54:6069
21	D17S800	4	0	0	Breast	HG 94:231
21	D17S902	37	10	0.27	Breast	CR 54:2549
21	D17S902	16	4	0.25	Prostate	GCC 13:278
21	D17S579	1	0	0	Bladder	HG 94:231
21	D17S579	19	11	0.58	Breast	CR 52:2624

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21	D17S579	23	5	0.22	Breast	CR 54:6068
21	D17S579	34	7	0.21	Breast	O 8:781
21	D17S579	85	20	0.24	Breast	GCC 11:58
21	D17S579	16	5	0.31	Breast	AJOG 172:908
21	D17S579	90	12	0.13	Breast	CR 54:2549
21	D17S579	4	1	0.25	Breast	HG 94:231
21	D17S579	52	21	0.4	Breast	BCRT 32:5
21	D17S579	14	4	0.29	Esophageal	CL 97:129
21	D17S579	26	8	0.31	Head&Neck	CR 54:1352
21	D17S579	17	13	0.76	Ovary	AJOG 172:908
21	D17S579	23	9	0.39	Ovary	GO 65:265
21	D17S579	2	0	0	Ovary	HG 94:231
21	D17S579	18	14	0.78	Ovary	IJC 54:220
21	D17S579	37	22	0.59	Ovary	CR 56:606
21	D17S579	19	14	0.74	Ovary	IJC 54:585
21	D17S579	20	2	0.1	Prostate	CR 55:1002
21	D17S579	20	2	0.1	Prostate	O 11:1241
21	D17S579	25	0	0	Uterus	CR 54:4294
Unknown	D17S609	75	18	0.24	Breast	BR 59:4056
Unknown	D17S509	26	3	0.12	Breast	HG 91:6
Unknown	D17S609	11	5	0.45	Colon	CR 54:85
21	HOX2	19	1	0.05	Prostate	O 11:1241
Unknown	PPY	20	5	0.25	Breast	CR 53:5617
Unknown	D17S806	26	2	0.08	Cervix	CR 56:197
21.3-22	COL1A1	24	10	0.42	Breast	O 8:781
22	D17S41	43	21	0.49	Breast	CR 53:5617
12.0-24	D17S41	20	8	0.4	Breast	O 8:781
22	D17S41	11	7	0.64	Ovary	IJC 54:85
12.0-24	D17S41	20	5	0.25	Ovary	IJC 54:546
12.0-24	D17S41	8	7	0.88	Ovary	IJC 54:220
21.3-22	NM23	23	6	0.26	Breast	GCC 1:113
21.3-22	NM23	61	8	0.13	Breast	ANYAS p.137
21.3-22	NM23	29	3	0.1	Colon	CR 58:3979
21.3-22	NM23	17	3	0.18	Colon	EJC 30A:664
21.3-22	NM23	7	0	0	Melanoma	GCC 1:169
21.3-22	NM23	20	13	0.65	Ovary	IJC 54:85
21.3-22	NM23	21	2	0.09	Stomach	JJCP 84:184
21.3-22	NM23	7	0	0	Uterus	C 73:1686
Unknown	NME1	55	25	0.45	Breast	CR 53:5617
Unknown	NME1	68	20	0.29	Breast	GCC 11:58
Unknown	NME1	47	15	0.32	Breast	CR 52:2624
Unknown	NME1	45	10	0.22	Breast	BCRT 28:231
Unknown	NME1	48	7	0.15	Breast	JJCP 84:1159
Unknown	NME1	18	1	0.06	Cervix	CR 54:4481
Unknown	NME1	27	2	0.07	Esophageal	C 73:2472
Unknown	NME1	27	2	0.07	Head&Neck	C 73:2472

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Unknown	NME1	17	6	0.35	Ovary	IJC 54:220
Unknown	NME1	21	1	0.05	Prostate	JU 151:1073
Unknown	NME1	21	8	0.38	Testis	O 9:2245
Unknown	NME1	18	8	0.44	Testis	O 9:2245
Unknown	D17S74	16	2	0.13	Breast	BCRT 28:231
22	D17S74	50	10	0.2	Breast	BCRT 28:231
22	D17S74	8	2	0.25	Breast	CR 54:4801
22	D17S74	67	13	0.19	Breast	HG 91:6
Unknown	D17S74	92	3	0.03	Breast	CR 54:4200
22	D17S74	106	49	0.46	Breast	CR 54:4200
Unknown	D17S74	95	20	0.21	Breast	CR 54:4200
23	D17S74	49	12	0.24	Breast	CR 53:3382
Unknown	D17S74	76	22	0.29	Breast	JJCR 84:1159
Unknown	D17S74	57	10	0.18	Breast	JJCR 84:1159
23	D17S74	53	20	0	Esophageal	GCC 10:177
Unknown	D17S74	54	20	0.37	Esophageal	GCC 10:177
Unknown	D17S74	290	3	0.01	Esophageal	CR 54:2998
Unknown	D17S74	30	3	0.1	Kidney	CR 51:820
Unknown	D17S74	74	2	0.03	Liver	CR 53:891
Unknown	D17S74	12	2	0.17	Liver	CR 53:368
22	D17S74	43	7	0.16	Lung	CR 47:5130
22	D17S74	9	8	0.89	Lung	PN 86:5099
22	D17S74	3	3	0.33	Lung	PN 86:5099
22	D17S74	11	2	0.18	Lung	PN 86:5099
Unknown	D17S74	39	8	0.21	Lung	CR 52:2478
Unknown	D17S74	24	10	0.42	Ovary	IJC 54:546
Unknown	D17S74	23	16	0.7	Ovary	IJC 54:220
Unknown	D17S74	26	10	0.38	Ovary	CR 51:5118
23	D17S74	6	0	0	Ovary	CR 53:3382
23	D17S74	8	1	0.12	Ovary	CR 53:3382
22	D17S74	10	2	0.2	Ovary	IJC 52:573
23	D17S74	17	6	0.35	Ovary	CR 53:3382
23	D17S74	10	2	0.2	Ovary	CR 53:3382
22	D17S74	17	12	0.71	Ovary	IJC 54:85
Unknown	D17S74	98	4	0.22	Sarcoma	CR 49:6747
Unknown	D17S74	22	3	0.14	Sarcoma	CR 52:2419
Unknown	MPO	11	4	0.36	Breast	CR 52:2624
Unknown	MPO	31	5	0.16	Head&Neck	O 9:2077
Unknown	MPO	20	1	0.05	Prostate	O 11:241
Unknown	D17S86	44	9	0.2	Breast	CR 53:5617
21.1-21.2	C117-24	36	13	0.36	Esophageal	CR 54:1636
12-21.1	C117-316	37	11	0.3	Breast	CR 53:3382
12-21.1	C117-316	32	9	0.28	Esophageal	CR 54:1636
12-21.1	C117-316	13	6	0.46	Ovary	CR 53:3382
12-21.1	C117-316	1	0	0	Ovary	CR 53:3382
12-21.1	C117-316	9	1	0.11	Ovary	CR 53:3382

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12-21.1	CI17-316	3	0	0	Ovary	CR 53:3382
21.3	CI17-477	32	22	0.69	Esophageal	CR 54:1638
21.3	CI17-26	7	3	0.43	Esophageal	CR 54:1638
21.3	CI17-28	26	15	0.58	Esophageal	CR 54:1638
21.3	C117-592	18	8	0.44	Breast	CR 53:3382
21.3	C117-592	17	6	0.35	Esophageal	CR 54:1638
21.3	C117-592	4	2	0.5	Ovary	CR 53:3382
21.3	C117-592	1	0	0	Ovary	CR 53:3382
21.3	C117-592	3	2	0.67	Ovary	CR 53:3382
21.3	C117-592	1	0	0	Ovary	CR 53:3382
21.3	C117-701	138	48	0.35	Breast	CR 53:3382
21.3	C117-701	38	21	0.55	Esophageal	CR 54:1638
21.3	C117-701	12	5	0.42	Ovary	CR 53:3382
21.3	C117-701	7	0	0	Ovary	CR 53:3382
21.3	C117-701	15	9	0.6	Ovary	CR 53:3382
21.3	C117-701	12	2	0.17	Ovary	CR 53:3382
21.3	C117-730	96	36	0.38	Breast	CR 53:3382
21.3	C117-730	35	20	0.57	Esophageal	CR 54:1638
21.3	C117-730	4	0	0	Ovary	CR 53:3382
21.3	C117-730	4	0	0	Ovary	CR 53:3382
21.3	C117-730	12	6	0.5	Ovary	CR 53:3382
21.3	C117-730	4	2	0.5	Ovary	CR 53:3382
21.3	C117-507	25	7	0.28	Breast	CR 53:3382
21.3	C117-507	18	10	0.56	Esophageal	CR 54:1638
21.3	C117-507	3	1	0.33	Ovary	CR 53:3382
21.3	C117-507	5	2	0.4	Ovary	CR 53:3382
21.3	C117-507	7	6	0.86	Ovary	CR 53:3382
21.3	C117-507	3	1	0.33	Ovary	CR 53:3382
21.3	C117-533	93	25	0.27	Breast	CR 53:3382
21.3	C117-533	42	21	0.5	Esophageal	CR 54:1638
21.3	C117-533	9	4	0.44	Ovary	CR 53:3382
21.3	C117-533	9	3	0.33	Ovary	CR 53:3382
21.3	C117-533	11	6	0.55	Ovary	CR 53:3382
21.3	C117-533	7	1	0.14	Ovary	CR 53:3382
21-23	D17S78	14	0	0	Brain	AJP 145:1175
21-23	D17S78	25	5	0.2	Ovary	IJC 54:546
22-24	GH	39	13	0.33	Breast	O 8:781
22-24	GH	16	4	0.25	Breast	CR 52:2624
22-24	GH	59	13	0.22	Breast	CR 53:5617
22-24	GH	12	1	0.08	Lung	CR 49:5130
22-24	GH	14	7	0.5	Ovary	GO 55:245
22-24	GH	15	1	0.07	Uterus	CR 51:5632
Unknown	66/E6	11	4	0.36	Breast	O 8:781
23-24	D17S40	23	10	0.43	Breast	CR 53:5617
Unknown	D17S40	14	5	0.36	Breast	O 8:781
23-24	D17S40	15	9	0.6	Ovary	IJC 54:85

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Unknown	D17S10	18	4	0.22	Ovary	IJC 54:546
23-qter	D17S21	15	0	0	Brain	AJP 145:1175
23-qter	D17S21	20	7	0.35	Breast	CR 53:5617
23-qter	D17S21	25	13	0.52	Ovary	IJC 54:546
Unknown	D17S515	32	6	0.19	Head&Neck	O 9:2077
Unknown	D17S801	32	4	0.12	Cervix	CR 56:197
Unknown	D17S785	37	1	0.03	Head&Neck	CR 54:4756
Unknown	D17S785	37	16	0.43	Head&Neck	CR 54:4756
Unknown	D17S785	6	3	0.5	Kidney	GCC 12:76
Unknown	D17S785	27	1	0.04	Melanoma	CR 56:589
Unknown	CACNLB1	19	2	0.11	Prostate	O 11:1241
Unknown	D17S20	72	5	0.07	Breast	CR 53:5617
23-25.5	D17S4	9	0	0	Brain	CR 49:6572
23-25.5	D17S4	14	3	0.21	Brain	CR 49:6572
23-25.5	D17S4	34	1	0.03	Brain	AJP 145:1175
23-25.5	D17S4	47	6	0.13	Breast	HG 91:6
23-25.4	D17S4	42	10	0.23	Breast	BJC 69:754
23-25.3	D17S4	51	21	0.41	Breast	CR 54:4200
23-25.3	D17S4	34	10	0.29	Breast	IJC 53:11
23-25.3	D17S4	104	28	0.27	Breast	CR 51:5794
23-25.5	D17S4	63	24	0.38	Breast	CR 53:5617
23-25.3	D17S4	34	10	0.29	Breast	GCC 4:113
23-25.5	D17S4	47	16	0.34	Breast	Lan 336:761
23-25.3	D17S4	36	7	0.19	Breast	ANYAS p.137
23-25.5	D17S4	35	3	0.09	Cervix	CR 54:4481
23-25	D17S4	13	0	0	Cervix	BJC 67:71
23-25.3	D17S4	20	3	0.15	Colon	JNCI 84:1100
23-25.3	D17S4	23	0	0	Colon	CCG 48:167
23-25.5	D17S4	25	5	0.2	Colon	CR 50:7166
23-25.5	D17S4	14	1	0.07	Esophageal	CR 51:2113
23-25.3	D17S4	23	7	0.3	Esophageal	CR 54:2996
23-25.5	D17S4	14	1	0.07	Kidney	CR 51:1071
23-25.5	D17S4	8	2	0.25	Liver	CR 53:368
23-25.3	D17S4	5	0	0	Liver	PNAS 86:8852
23-25.3	D17S4	2	0	0	Lung	CR 49:5130
23-25.3	D17S4	16	11	0.69	Ovary	O 7:2069
23-25.3	D17S4	16	2	0.12	Ovary	O 7:2069
23-25.3	D17S4	41	30	0.73	Ovary	O 7:2069
23-25.3	D17S4	7	4	0.57	Ovary	Unknown
23-25.3	D17S4	29	11	0.38	Ovary	IJC 54:546
23-25.3	D17S4	21	2	0.1	Ovary	CR 51:5118
23-25.3	D17S4	30	11	0.37	Ovary	IJC 52:575
23-25	D17S4	15	10	0.67	Ovary	IJC 54:85
23-25.5	D17S4	15	10	0.67	Ovary	IJC 54:85
23-25.3	D17S4	19	12	0.63	Ovary	IJC 54:220
23-25	D17S4	4	0	0	Pancreas	CR 54:2761

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23-25	D17S4	11	0	0	Prostate	GCC 11:119
23-25	D17S4	9	2	0.22	Sarcoma	CR 52:2419
23-25.5	D17S4	12	9	0.75	Sarcoma	CR 52:2419
23-25.3	D17S4	14	3	0.21	Sarcoma	CR 49:6247
23-25	D17S4	7	0	0	Stomach	CR 51:2926
23-25.5	D17S4	42	17	0.4	Testis	O 9:2245
23.3-25.3	TK1	21	1	0.05	Breast	CR 53:5617
23-qter	D17S77	31	2	0.06	Brain	AJP 145:1175
23-qter	D17S77	30	11	0.37	Breast	CR 53:5617
Unknown	D17S26	9	0	0	Breast	CR 53:5617
Unknown	D17S26	16	5	0.31	Ovary	CR 50:2724
23-25	D17S75	71	23	0.32	Breast	CR 51:5794
25-25.3	D17S24	23	0	0	Brain	AJP 145:1175
Unknown	D17S24	34	12	0.35	Breast	GCC 4:113
Unknown	D17S24	59	27	0.46	Breast	CR 54:5617
Unknown	D17S24	59	20	0.34	Breast	O 8:781
23-25.3	D17S24	40	17	0.42	Breast	CR 54:4200
23-25	D17S24	42	10	0.24	Breast	CR 51:5794
23-25.3	D17S24	40	17	0.42	Breast	CR 54:4200
23-25.3	D17S24	20	8	0.4	Breast	GCC 2:191
23-25.3	D17S24	4	2	0.5	Breast	CR 53:3804
Unknown	D17S24	21	2	0.1	Colon	JNCI 84:1100
23-25.3	D17S24	18	11	0.61	Ovary	IJC 54:85
Unknown	D17S24	16	8	0.5	Ovary	IJC 54:546
23-25.3	D17S24	18	11	0.61	Ovary	IJC 54:85
23-25	D17S24	3	0	0	Ovary	CR 51:5118
Unknown	D17S24	9	1	0.11	Prostate	G 11:530
23-25	D17S27	17	6	0.35	Breast	CR 51:5794
Unknown	D17S79	9	2	0.22	Breast	CR 53:5617
Unknown	D17S79	9	2	0.22	Breast	CR 53:5617
Unknown	D17S587	1	0	0	Bladder	HG 94:231
12.0-21	D17S588	1	0	0	Bladder	HG 94:231
Unknown	Unknown	28	3	0.11	Brain	CR 50:5704
25.1	Unknown	31	9	0.29	Breast	CR 53:3382
23	Unknown	31	10	0.32	Breast	CR 53:3382
22	Unknown	41	14	0.34	Breast	CR 53:3382
25.3	Unknown	45	13	0.29	Breast	CR 53:3382
21	D173700	54	10	0.19	Breast	CR 54:2549
21	D17S1184	11	2	0.18	Breast	CR 54:6069
21	D17S1322	11	10	0.91	Breast	CR 54:6069
21	D17S1325	11	11	1	Breast	CR 54:6069
21	D17S1328	6	5	0.83	Breast	CR 54:6069
21	D17S183	36	8	0.22	Breast	CR 54:2549
Unknown	D17S2	4	0	0	Breast	GCC 2:191
Unknown	D17S293	15	3	0.2	Breast	AJOG 172:908
Unknown	D17S308	23	9	0.39	Breast	O 8:781

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Unknown	D17S5-D17S1-D17S31-D17S509-D17S74-D17S4	75	18	0.24	Breast	CR 53:3707
Unknown	D17S587	6	1	0.17	Breast	HG 94:231
12.0-21	D17S588	9	2	0.22	Breast	O 8:791
12.0-21	D17S588	6	1	0.17	Breast	HG 94:231
12.0-21	D17S588	17	8	0.47	Breast	AJOG 172:908
21	D17S648	39	7	0.18	Breast	CR 54:2549
Unknown	D17S68	25	16	0.7	Breast	CR 54:4200
21	D17S702	92	21	0.23	Breast	CR 54:2549
Unknown	D17S702	80	24	0.3	Breast	GCC 11:58
Unknown	D17S733	65	18	0.28	Breast	GCC 11:58
21	D17S746	36	10	0.28	Breast	CR 54:2549
21	D17S750	59	14	0.24	Breast	CR 54:2549
23-qter	D17S77	30	11	0.37	Breast	CR 53:5617
Unknown	D17S773	9	2	0.22	Breast	CR 53:5617
21	D17S776	10	6	0.6	Breast	CR 54:6069
21	D17S776	70	17	0.24	Breast	GCC 11:58
21	D17S776	63	19	0.3	Breast	CR 54:2549
21	D17S846	74	24	0.32	Breast	CR 54:2549
21	D17S855	30	8	0.27	Breast	CR 54:2549
21	D17S855	86	21	0.24	Breast	GCC 11:58
21	D17S855	10	8	0.8	Breast	CR 54:6069
21	D17S856	53	10	0.19	Breast	CR 54:2549
21	D17S857	68	17	0.25	Breast	CR 54:2549
21	D17S859	17	2	0.12	Breast	CR 54:2549
21	D17S870	441	173	0.39	Breast	BJC 71:436
21	D17S870-CI17-730	289	98	0.34	Breast	C 74:2281
Unknown	EDH17B-H9D-A3T	19	7	0.37	Breast	GCC 11:58
Unknown	EDH17B-HSD-DEL	20	9	0.45	Breast	GCC 11:58
Unknown	EFB3	15	6	0.4	Breast	CR 53:5617
21	GAS	50	13	0.26	Breast	CR 54:2549
Unknown	PROHIB	6	1	0.17	Cervix	GCC 9:119
Unknown	D17S791	22	1	0.05	Endocrine	CR 56:599
25.3	Unknown	40	11	0.28	Esophageal	CR 54:1638
22	Unknown	33	16	0.48	Esophageal	CR 54:1638
25.1	Unknown	26	14	0.54	Esophageal	CR 54:1638
Unknown	D17S874	35	20	0.57	Esophageal	GCC 10:177
Unknown	GP3A	15	6	0.4	Head&Neck	O 9:2077
12.0-21	D17S588	34	2	0.06	Kidney	BJC 69:230
Unknown	D17S-B02-B05-B09	22	5	0.23	Leukemia	CR 55:5377
Unknown	D17S32	13	0	0	Liver	CR 53:368
25.3	Unknown	7	3	0.43	Ovary	CR 53:3382
22	Unknown	3	1	0.33	Ovary	CR 53:3382
25.1	Unknown	7	0	0	Ovary	CR 53:3382
25.1	Unknown	17	6	0.35	Ovary	CR 53:3382
22	Unknown	3	0	0	Ovary	CR 53:3382

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25.3	Unknown	8	3	0.38	Ovary	CR 53:3382
25.3	Unknown	8	4	0.5	Ovary	CR 53:3382
22	Unknown	5	4	0.8	Ovary	CR 53:3382
25.3	Unknown	6	0	0	Ovary	CR 53:3382
22	Unknown	1	0	0	Ovary	CR 53:3382
23	Unknown	3	0	0	Ovary	CR 53:3382
23	Unknown	5	5	1	Ovary	CR 53:3382
25.1	Unknown	11	6	0.55	Ovary	CR 53:3382
25.1	Unknown	10	1	0.1	Ovary	CR 53:3382
23	Unknown	2	0	0	Ovary	CR 53:3382
23	Unknown	8	3	0.38	Ovary	CR 53:3382
Unknown	46E6-HOX2B-D17S250-588-579	18	10	0.56	Ovary	BJC 72:1330
Unknown	D17S136	6	5	0.83	Ovary	IJC 54:220
Unknown	D17S174	10	8	0.8	Ovary	IJC 54:220
Unknown	D17S180	6	4	0.67	Ovary	IJC 54:220
Unknown	D17S250-579-588-HM23-GH	120	64	0.53	Ovary	CR 53:1216
12.0-21	D17S250-THRA1-D17S846-D17S856-D17S855-D17S183-D17S579-D17S588	3	2	0.67	Ovary	AJHG 55:666
12.0-21	D17S250-THRA1-D17S846-D17S856-D17S855-D17S183-D17S579-D17S588	14	12	0.86	Ovary	AJHG 55:666
12.0-21	D17S250-THRA1-D17S846-D17S856-D17S855-D17S183-D17S579-D17S588	11	8	0.73	Ovary	AJHG 55:666
12.0-21	D17S250-THRA1-D17S846-D17S856-D17S855-D17S183-D17S579-D17S588	1	1	1	Ovary	AJHG 55:666
Unknown	D17S293	11	9	0.82	Ovary	IJC 54:220
Unknown	D17S293	18	14	0.78	Ovary	AJOG 172:908
Unknown	D17S308	17	14	0.82	Ovary	IJC 54:220
Unknown	D17S582	2	0	0	Ovary	HG 94:231
12.0-21	D17S588	11	6	0.55	Ovary	BJC 69:429
12.0-21	D17S588	20	14	0.7	Ovary	AJOG 172:908
12.0-21	D17S588	2	0	0	Ovary	HG 94:231
Unknown	D17S73-41 q-77	37	28	0.76	Ovary	CR 53:2993
22-23	NME1-D17S74-GH-D17S40-D17S4-D17S75	11	11	1	Ovary	AJHG 55:666
22-23	NME1-D17S74-GH-D17S40-D17S4-D17S75	3	3	1	Ovary	AJHG 55:666

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22-23	NME1-D17S74-GH-D17S40-D17S4-D17S75	1	1	1	Ovary	AJHG 55:666
22-23	NME1-D17S74-GH-D17S40-D17S4-D17S75	14	14	1	Ovary	AJHG 55:666
Unknown	D17S1323	12	3	0.25	Prostate	O 11:1241
Unknown	D17S1327	15	2	0.13	Prostate	O 11:1241
12.0-21	D17S588	19	2	0.11	Prostate	CR 55:1002
12.0-21	D17S588	19	2	0.11	Prostate	O 11:1241
21.3	D17S752	14	1	0.07	Prostate	GCC 13:278
21	D17S776	12	5	0.42	Prostate	O 11:1241
21	D17S846	19	2	0.11	Prostate	O 11:1241
21	D17S855	18	8	0.44	Prostate	O 11:1241
21	D17S855	18	8	0.44	Prostate	CR 55:1002
21	D17S856	15	5	0.33	Prostate	O 11:1241
21	D17S856	15	6	0.4	Prostate	CR 55:1002
21	D17S857	20	2	0.1	Prostate	O 11:1241
21	D17S859	18	1	0.06	Prostate	O 11:1241
Unknown	KRT9	18	2	0.11	Prostate	O 11:1241
Unknown	D17S32	10	1	0.1	Sarcoma	CR 49:6247
Unknown	D17S32	14	2	0.14	Sarcoma	CR 52:2419
Unknown	D17S293	19	0	0	Uterus	CR 54:4294
Unknown	PROHIB	2	1	0.5	Uterus	GCC 9:119
SUM		9605	3006	0.31		

Chromosome 18 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
11.2-12.1	TTR	18	9	0.5	Colon	IJC 53:382
11.1-11.2	D18S7	5	2	0.4	Breast	CR 53:3804
11.1-11.2	D18S7	7	2	0.29	Colon	S 241:961
11.1-11.2	D18S7	9	2	0.22	Stomach	HG 92:244
11.1-11.2	D18S7	17	8	0.47	Stomach	CR 52:3099
Unknown	D18S1	7	1	0.14	Breast	GCC 2:191
Unknown	D18S1	8	4	0.5	Colon	IJC 53:382
Unknown	D18S1	11	0	0	Colon	N 331:273
Unknown	D18S1	16	4	0.25	Colon	CR 50:7166
Unknown	D18S1	1	1	1	Lung	PNAS 86:5099
Unknown	D18S1	5	2	0.4	Lung	PNAS 86:5099
Unknown	D18S1	4	1	0.25	Lung	PNAS 86:5099
Unknown	D18S1	9	3	0.33	Ovary	Q 7:1059
Unknown	D18S1	15	7	0.47	Sarcoma	CR 52:2419
Unknown	D18S1	6	2	0.33	Uterus	CR 51:5632
11	D18S6	8	2	0.25	Bladder	BJC 70:697
11	D18S6	12	2	0.17	Breast	PNAS 87:7737
11-pter	D18S6	24	5	0.21	Breast	JNCI 84:506
11	D18S6	16	6	0.38	Cervix	CR 54:4481
11	D18S6	19	9	0.47	Colon	CR 50:7166
11	D18S6	6	0	0	Colon	CCG 10:167
11	D18S6	17	3	0.18	Ovary	IJC 54:546
11	D18S6	1	0	0	Prostate	JU 151:1073
11	D18S6	15	4	0.27	Testis	O 9:2245
11	D18S6	5	1	0.2	Testis	GCC 13:249
Unknown	D18S57	33	10	0.3	Cervix	CR 56:197
Unknown	D18S22	14	2	0.14	Brain	CR 50:5784
Unknown	D18S22	17	3	0.18	Breast	GCC 2:191
Unknown	D18S22	29	11	0.38	Esophageal	CR 54:2996
Unknown	D18S22	11	7	0.64	Sarcoma	CR 52:2419
21.3	D18S8	7	3	0.43	Breast	CR 53:3804
21.3	D18S8	27	9	0.33	Colon	S 241:961
21.3	D18S8	7	5	0.71	Stomach	CR 52:3099
21.3	D18S8	14	6	0.43	Stomach	HG 92:244
Unknown	D18S24	13	1	0.08	Breast	CR 50:7184
Unknown	D18S24	6	0	0	Cervix	GCC 9:119
Unknown	D18S24	4	0	0	Kidney	CR 51:820
Unknown	D18S24	17	4	0.24	Lung	CR 52:2478
Unknown	D18S24	8	0	0	Ovary	CR 51:5118
Unknown	D18S24	3	0	0	Uterus	GCC 9:119
11.2-12.1	PALB	18	9	0.5	Colon	CR 50:7166
11.2-12.1	PALB	11	2	0.18	Colon	GCC 3:468
11.2-12.1	PALB	6	0	0	Pancreas	GCC 3:468
11.2-12.1	PALB	8	2	0.25	Stomach	GCC 3:468
11.2-12.1	PALB	3	0	0	Uterus	CR 51:5632
21.3	DCC	28	8	0.29	Bladder	CR 55:5213

Chromosome 18 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
11.21-PTER	D18S40	25	3	0.12	Uterus	CR 54:4294
Unknown	Unknown	12	1	0.08	Brain	CR 50:5784
Unknown	D18S16	23	0	0	Breast	CR 53:4356
11.3	D18S3	9	1	0.11	Breast	CR 50:7184
Unknown	D18S53	31	8	0.26	Cervix	CR 56:1097
Unknown	D18S59	20	1	0.05	Endocrine	CR 56:599
Unknown	D18S21	20	2	0.1	Esophageal	CR 54:4896
Unknown	D18S21	15	1	0.07	Esophageal	CR 51:2113
Unknown	D18S3	18	2	0.11	Esophageal	GCC 10:177
11.21-PTER	D18S40	22	6	0.27	Head&Neck	CR 54:1152
Unknown	D18S59	13	0	0	Head&Neck	CR 54:4756
Unknown	D18S59	18	3	0.17	Head&Neck	CR 54:4756
11.3	D18S3	12	0	0	Kidney	CR 51:820
Unknown	D18S59	21	0	0	Kidney	PNAS 92:2854
Unknown	D18S59	6	1	0.17	Kidney	PNAS 92:7854
Unknown	D18S54	19	1	0.05	Leukemia	CR 55:5377
11.3	D18S3	16	4	0.25	Lung	CR 52:2478
Unknown	D18S59	33	4	0.12	Melanoma	CR 56:589
11.3	D18S3	6	0	0	Ovary	CR 51:5110
11.21-PTER	D18S40	15	4	0.27	Ovary	BJC 72:1330
Unknown	D18S6	10	1	0.1	Ovary	CR 53:2563
11.3	D18S3	15	0	0	Prostate	G 11:530
Unknown	D18S21	10	2	0.2	Sarcoma	CR 52:2419
11.21-PTER	D18S40	25	3	0.12	Uterus	CR 54:4294
SUM		386	45	0.12		

Chromosome 18 - q Arm

21.3	DCC	15	8	0.53	Bladder	BJC 70:697
21.3	DCC	26	2	0.08	Breast	CR 53:4356
21.3	DCC	16	5	0.31	Breast	BJC 68:66
21	DCC	5	1	0.2	Cervix	BJC 67:71
21.3	DCC	12	3	0.25	Cervix	BJC 67:71
21.3	DCC	48	18	0.38	Colon	EJC 30A:664
21.3	DCC	25	13	0.52	Colon	CR 54:3979
21.3	DCC	4	1	0.25	Colon	O 9:991
21.3	DCC	41	29	0.71	Colon	S 247:49
21.3	DCC	19	0	0	Endocrine	GCC 13:9
21.3	DCC	44	10	0.23	Esophageal	CR 54:3007
21.3	DCC	50	12	0.24	Esophageal	CR 52:6525
21.3	DCC	5	1	0.2	Kidney	GCC 12:76
21.3	DCC	19	11	0.58	Leukemia	B 83:3449
21.3	DCC	26	8	0.31	Leukemia	B 82:927
21.3	DCC	9	3	0.33	Leukemia	B 82:927
21.3	DCC	11	1	0.09	Liver	CR 51:589
21.3	DCC	6	2	0.33	Ovary	BJC 71:462
21.3	DCC	34	15	0.44	Ovary	O 7:1059
21.3	DCC	7	3	0.43	Ovary	O 7:1059
21.3	DCC	2	2	1	Pancreas	CR 54:2761
21	DCC	12	2	0.17	Prostate	PNAS 87:8751
21.3	DCC	11	5	0.45	Prostate	CR 53:2723
21.3	DCC	13	5	0.38	Prostate	GCC 11:119
21.3	DCC	12	2	0.17	Prostate	CSurveys 11:1
21	DCC	7	5	0.71	Stomach	CR 52:3099
21.3	DCC	18	5	0.28	Stomach	LI 74:835
21.3	DCC	10	5	0.5	Stomach	CR 52:3099
21.3	DCC	51	17	0.33	Uterus	CR 54:4294
21.3	DCC	8	1	0.12	Uterus	CR 51:5632
21.3	DCC	5	1	0.2	Uterus	CR 51:5633
21.2-21.3	D18S35	22	0	0	Uterus	CR 54:4294
21.3	BCL2	14	1	0.07	Breast	PNAS 87:7737
21.3	BCL2	10	6	0.6	Colon	JJCR 85:584
21.3	BCL2	20	10	0.5	Ovary	O 7:1059
21.3	BCL2	7	2	0.29	Prostate	GCC 11:119
21.3	BCL2	17	4	0.24	Stomach	JJCR 85:584
Unknown	D18S68	23	8	0.35	Cervix	CR 56:197
Unknown	D18S19	22	9	0.41	Breast	PNAS 88:7737
Unknown	D18S19	8	3	0.38	Prostate	GCC 11:119
21.3-qter	D18S5	9	4	0.44	Bladder	BJC 70:697
12	D18S5	17	4	0.24	Bladder	CR 51:5405
21.3-qter	D18S5	70	11	0.16	Breast	JJCR 84:1159
12	D18S5	5	1	0.2	Breast	GCC 2:191
21.3-qter	D18S5	43	6	0.14	Breast	AJP 140:215
21.3-qter	D18S5	16	11	0.69	Breast	PNAS 87:7737

Chromosome 18 - q Arm

21.3-qter	D18S5	21	2	0.1	Cervix	CR 54:4481
12	D18S5	7	0	0	Cervix	CR 49:3598
21.3-qter	D18S5	6	2	0.33	Colon	O 9:991
21.3-qter	D18S5	21	16	0.76	Colon	IJC 53:382
12	D18S5	19	12	0.63	Colon	CR 50:7166
12	D18S5	29	11	0.38	Esophageal	GCC 10:177
12	D18S5	19	1	0.05	Kidney	CR 51:1544
12	D18S5	18	1	0.06	Liver	JJCR 81:108
12	D18S5	28	3	0.11	Lung	PN 84:9252
12	D18S5	7	0	0	Neuroblastoma	CR 49:1095
21.3-qter	D18S5	16	4	0.25	Ovary	IJC 54:546
21.3-qter	D18S5	15	9	0.6	Ovary	O 7:1059
21.3-qter	D18S5	21	12	0.57	Prostate	JU 151:1073
21.3-qter	D18S5	16	4	0.25	Prostate	GCC 11:119
12	D18S5	13	0	0	Stomach	CR 46:2988
21.3-qter	D18S5	15	10	0.67	Stomach	CR 52:3099
21.3-qter	D18S5	14	1	0.07	Testis	GCC 13:249
12	D18S5	42	16	0.38	Testis	O 9:2245
12	D18S5	9	2	0.22	Uterus	CR 51:5632
Unknown	D18S58-D18S61	6	1	0.17	Kidney	PNAS 92:2854
Unknown	D18S58-D18S61	22	0	0	Kidney	PNAS 92:2854
23	D18S11	67	17	0.25	Breast	PNAS 87:7737
23	D18S11	8	3	0.38	Colon	GCC 3:468
23	D18S11	25	8	0.32	Ovary	IJC 54:546
23	D18S11	35	21	0.6	Ovary	O 7:1059
23	D18S11	5	0	0	Pancreas	GCC 3:468
23	D18S11	13	2	0.15	Prostate	GCC 11:119
23	D18S11	13	2	0.15	Stomach	GCC 3:468
Unknown	D18S70	41	0	0	Head&Neck	CR 54:4756
Unknown	D18S70	43	3	0.07	Head&Neck	CR 54:4756
Unknown	D18S70	21	0	0	Kidney	PNAS 92:2854
Unknown	D18S70	6	1	0.17	Kidney	PNAS 92:2854
Unknown	D18S70	23	5	0.22	Melanoma	CR 56:589
Unknown	D18S70	23	5	0.22	Melanoma	CR 56:589
12.1-21.1	Unknown	18	4	0.22	Bladder	BJC 70:697
23	Unknown	11	4	0.36	Bladder	BJC 70:697
Unknown	D18S22	12	0	0	Brain	CR 49:6572
Unknown	D18S46	17	1	0.06	Endocrine	CR 56:599
Unknown	D18S34	26	6	0.23	Head&Neck	CR 54:1152
Unknown	D18S:58-67	23	4	0.17	Leukemia	CR 55:5377
Unknown	Unknown	2	0	0	Liver	BJC 67:1007
Unknown	Unknown	5	0	0	Liver	BJC 64:1083
Unknown	DCC-D18S34	28	12	0.43	Ovary	CR 53:2393
Unknown	MBP- D18S:34-35	15	6	0.4	Ovary	BJC 72:1330
Unknown	PLRNH2	7	2	0.29	Ovary	O 7:1059
Unknown	Unknown	6	4	0.67	Pancreas	CR 54:2761

Chromosome 18 - q Arm

Unknown	Unknown	1	0	0	Pancreas	CR 54:2761
Unknown	Unknown	6	0	0	Pancreas	BJC 65:809
23	Unknown	2	2	1	Prostate	JU 151:1073
Unknown	D18S31	19	2	0.11	Testis	GCC 13:249
Unknown	JOSH4.4	20	5	0.25	Testis	O 9:2245
SUM		2301	659	0.29		

Chromosome 19 - p Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
Unknown	LIPE	21	0	0	Uterus	CR 54:4294
13.2-CEN	D19S11	36	2	0.06	Brain	AJP 145:1175
Unknown	D19S20	12	0	0	Brain	CR 50:5784
Unknown	D19S20	35	1	0.03	Brain	AJP 145:1175
Unknown	D19S20	8	0	0	Brain	CR 49:6572
13.2	D19S24	15	0	0	Brain	AJP 145:1175
12-13.2	D19S76	14	0	0	Brain	CR 54:1397
12-13.2	D19S76	11	1	0.09	Brain	CR 54:1397
13.2-13.1	LDLR	3	1	0.33	Brain	CR 54:1397
13.2-13.1	LDLR	11	0	0	Brain	CR 54:1397
13.2-CEN	D19S11	26	7	0.27	Breast	CR 53:4356
Unknown	D19S20	36	7	0.19	Breast	CR 50:7184
13.3-.2	D19S22	35	1	0.03	Breast	CR 53:4356
13.2-CEN	D19S11	45	1	0.02	Cervix	CR 54:4481
13.3	D19S177	27	4	0.15	Cervix	CR 56:197
Unknown	D19S20	8	0	0	Cervix	GCC 9:119
Unknown	D19S721	29	7	0.24	Cervix	CR 56:197
Unknown	D19S7	26	4	0.15	Cervix	CR 54:4481
Unknown	D19S216	22	1	0.05	Endocrine	CR 56:599
Unknown	D19S20	22	6	0.27	Esophageal	CR 54:2996
Unknown	D19S20	25	2	0.08	Esophageal	GCC 10:177
13.3-.2	D19S22	34	11	0.32	Esophageal	GCC 10:177
13.3	D19S177	16	4	0.25	Head&Neck	CR 54:1152
Unknown	D19S216	15	0	0	Head&Neck	CR 54:4756
Unknown	D19S216	19	1	0.05	Head&Neck	CR 54:4756
Unknown	D19S221	19	6	0.32	Head&Neck	CR 54:1152
13.3	Unknown	48	7	0.15	Kidney	CR 51:5817
Unknown	D19S20	40	8	0.2	Kidney	CR 51:5817
Unknown	D19S20	25	8	0.32	Kidney	CR 51:820
13.3	D19S21	30	3	0.1	Kidney	CR 51:5817
Unknown	D19S216	3	0	0	Kidney	PNAS 92:2854
Unknown	D19S216	17	1	0.06	Kidney	PNAS 92:2854
13.2-TER	C3	3	0	0	Liver	CCG 48:92
13.3-.2	D19S22	28	1	0.04	Liver	CR 51:89
Unknown	D19S7	11	0	0	Liver	JJCR 81:108
Unknown	D19S20	26	3	0.12	Lung	CR 52:2478
Unknown	D19S7	17	0	0	Lung	PN 84:9152
Unknown	D19S216	25	2	0.08	Melanoma	CR 56:589
Unknown	Unknown	19	5	0.26	Ovary	CR 51:5118
13.2-CEN	D19S11	16	3	0.19	Ovary	IJC 54:546
13.2-CEN	D19S11	13	2	0.15	Ovary	CR 53:2393
13.3	D19S177	11	5	0.45	Ovary	BJC 69:429
Unknown	D19S20	13	5	0.38	Ovary	GO 55:198
Unknown	D19S20	24	8	0.33	Ovary	CR 51:5118
13.3-13.2	INSR	21	5	0.24	Ovary	IJC 54:546
13.3-.2	D19S22	6	0	0	Pancreas	CR 54:2761

Chromosome 19 - p Arm

13.2-CEN	D19S11	3	0	0	Prostate	G 11:530
Unknown	D19S20	21	5	0.24	Sarcoma	CR 52:2419
Unknown	D19S7	3	1	0.33	Sarcoma	CR 52:2419
13.2-CEN	D19S11	46	2	0.04	Testis	O 9:2245
Unknown	D19S20	20	1	0.05	Testis	LL 73:606
Unknown	D19S20	20	1	0.05	Testis	G 5:134
13.3-13.2	INSR	2	0	0	Testis	CCG 52:72
13.3-13.2	INSR	3	0	0	Testis	CCG 52:72
13.3-13.2	INSR	1	0	0	Testis	CCG 52:72
Unknown	D19S20	14	0	0	Uterus	GCC 9:119
Unknown	LIPE	21	0	0	Uterus	CR 54:4784
SUM		1099	143	0.13		

Chromosome 19 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
13.2	APOC2	11	0	0	Uterus	CR 54:4294
13.2	APOC2	33	19	0.58	Brain	AJP 145:1175
13.2	APOC2	22	8	0.36	Brain	CR 54:1397
13.2	APOC2	15	1	0.07	Brain	CR 54:1397
13.1-13.2	BCL3	5	4	0.8	Brain	CR 54:1397
13.1-13.2	BCL3	6	1	0.17	Brain	CR 54:1397
13.3	CKMM	34	19	0.56	Brain	AJP 145:1175
13.2	CYP2	24	13	0.54	Brain	AJP 145:1175
13.2	D19S178	12	1	0.08	Brain	CR 54:1397
13.2	D19S178	18	5	0.28	Brain	CR 54:1397
13.4	D19S180	21	9	0.43	Brain	CR 54:1397
13.4	D19S180	11	2	0.18	Brain	CR 54:1397
13.3	D19S191	23	6	0.26	Brain	CR 54:1397
13.1	D19S191	12	2	0.17	Brain	CR 54:1397
13.4	D19S22	18	1	0.06	Brain	CR 50:5784
13.4	D19S22	37	18	0.49	Brain	AJP 145:1175
12-13.1	D19S30	15	7	0.47	Brain	AJP 145:1175
12-13.1	D19S31	6	4	0.67	Brain	AJP 145:1175
13.1	D19S32	21	10	0.48	Brain	AJP 145:1175
13.1-13.2	D19S47	18	4	0.22	Brain	CR 54:1397
13.1-13.2	D19S47	11	2	0.18	Brain	CR 54:1397
12-13.1	D19S49	22	5	0.23	Brain	CR 54:1397
12-13.1	D19S49	12	1	0.08	Brain	CR 54:1397
13.3	D19S51	12	7	0.58	Brain	AJP 145:1175
13.3	D19S62	12	7	0.58	Brain	AJP 145:1175
13.3	D19S63	24	15	0.62	Brain	AJP 145:1175
12	D19S7	21	10	0.48	Brain	AJP 145:1175
11-CEN	D19S74	7	4	0.57	Brain	AJP 145:1175
12-13.1	D19S75	11	1	0.09	Brain	CR 54:1397
12-13.1	D19S75	19	3	0.16	Brain	CR 54:1397
13.2	D19S8	21	14	0.67	Brain	AJP 145:1175
Unknown	D19S9	6	2	0.33	Brain	AJP 145:1175
13.3	ERCC1	32	18	0.56	Brain	AJP 145:1175
13.3	ERCC2	16	7	0.44	Brain	AJP 145:1175
13.2	APOC2	25	2	0.08	Breast	GCC 2:191
13.4	D19S22	19	3	0.16	Breast	CR 50:7184
13.2	APOC2	29	3	0.1	Cervix	CR 56:197
Unknown	D19S223	24	3	0.12	Cervix	CR 56:197
Unknown	D19S9	1	0	0	Cervix	CR 49:3598
13.2	APOC2	17	1	0.06	Colon	CCG 48:167
12	D19S7	21	16	0.76	Colon	IJC 53:382
Unknown	D19S210	18	1	0.06	Endocrine	CR 56:599
13.1	D19S22	23	7	0.3	Esophageal	CR 54:2996
Unknown	D19S210	22	7	0.32	Head&Neck	CR 54:1152
Unknown	D19S255	10	0	0	Head&Neck	CR 54:4756
Unknown	D19S255	10	0	0	Head&Neck	CR 54:4756

Chromosome 19 - q Arm

Unknown	D19S210-D19S224	6	0	0	Kidney	PNAS 92:2854
Unknown	D19S210-D19S224	19	0	0	Kidney	PNAS 92:2854
13.4	D19S22	14	3	0.21	Kidney	CR 51:820
Unknown	D19S225	3	0	0	Kidney	PNAS 92:2854
Unknown	D19S225	17	1	0.06	Kidney	PNAS 90:2854
13.4	D19S22	24	11	0.46	Lung	CR 52:2478
13.4	D19S22	3	2	0.67	Lung	CR 52:2478
13.4	D19S22	1	1	1	Lung	CR 52:2478
13.4	D19S22	9	9	1	Lung	CR 52:2478
Unknown	D19S225	22	0	0	Melanoma	CR 56:589
12	D19S7	3	0	0	Neuroblastoma	CR 49:1095
Unknown	CYP1	7	1	0.14	Ovary	CR 50:2724
13.4	D19S22	16	4	0.25	Ovary	CR 51:5148
12-13.1	D19S49	13	3	0.23	Ovary	BJC 69:429
13.2	D19S8	23	5	0.22	Ovary	CR 51:5463
Unknown	D19S8-CYP2A	23	4	0.17	Ovary	CR 53:2393
13.2	D19S8	12	0	0	Prostate	G 11:530
13.4	D19S22	9	3	0.33	Sarcoma	CR 52:2419
12	D19S7	16	1	0.06	Stomach	CR 48:2980
12	D19S7	19	2	0.11	Testis	O 9:2245
13.2	APOC2	11	0	0	Uterus	CR 54:4294
SUM		1066	323	0.3		

Chromosome 20 - p Arm

Band	Marker	Total	Cases with LOH	LOH Frequency	Tumor Type	Reference
12	D20S6	4	1	0.25	Uterus	CR 51:5632
Unknown	Unknown	12	1	0.08	Brain	CR 50:5784
12	D20S6	8	0	0	Brain	CR 49:6577
Unknown	D20S19	6	0	0	Breast	CR 53:3804
Unknown	D20S19	87	2	0.05	Breast	CR 50:7104
12	D20S6	20	3	0.15	Breast	GCC 2:191
Unknown	D20S118	31	0	0	Cervix	CR 56:197
Unknown	D20S19	3	0	0	Cervix	GCC 9:119
12	D20S6	2	0	0	Cervix	CR 49:3598
12	D20S6	28	6	0.21	Cervix	CR 54:4481
Unknown	D20S98	16	2	0.12	Cervix	CR 56:197
Unknown	D20S95	16	0	0	Endocrine	CR 56:599
Unknown	D20S19	59	7	0.12	Esophageal	GCC 10:177
Unknown	D20S72	20	2	0.1	Esophageal	CR 54:2996
Unknown	D20S104	12	0	0	Head&Neck	CR 54:4756
Unknown	D20S104	23	2	0.09	Head&Neck	CR 54:4756
Unknown	D20S95	20	6	0.3	Head&Neck	CR 54:1152
Unknown	D20S104	17	1	0.06	Kidney	PNAS 92:2854
Unknown	D20S104	1	0	0	Kidney	PNAS 92:2854
Unknown	D20S117	5	0	0	Kidney	PNAS 92:2854
Unknown	D20S117	21	0	0	Kidney	PNAS 92:2854
Unknown	D20S19	29	1	0.03	Kidney	CR 51:820
Unknown	D20S19	39	0	0	Liver	CR 51:89
Unknown	D20S19	40	8	0.2	Lung	CR 52:2478
Unknown	D20S104	23	2	0.09	Melanoma	CR 56:589
12	D20S6	2	0	0	Neuroblastoma	CR 49:1095
Unknown	Unknown	16	0	0	Ovary	CR 53:2393
Unknown	D20S19	32	4	0.12	Ovary	CR 51:5118
12	D20S27	14	3	0.21	Ovary	EJC 69:429
12	D20S6	27	4	0.15	Ovary	IJC 54:546
Unknown	D20S19	5	0	0	Pancreas	CR 51:2761
12	D20S5	2	0	0	Pancreas	CR 54:2761
Unknown	D20S5	6	0	0	Prostate	G 11:530
Unknown	D20S19	8	2	0.25	Sarcoma	CR 52:2419
12	D20S5	13	4	0.31	Sarcoma	CR 52:2419
Unknown	D20S19	15	3	0.2	Stomach	CR 52:3099
12	D20S6	22	9	0.41	Testis	O 9:2245
Unknown	D20S19	2	0	0	Uterus	GCC 9:119
12	D20S27	26	0	0	Uterus	CR 54:4294
12	D20S6	4	1	0.25	Uterus	CR 51:5632
SUM		684	73	0.11		

Chromosome 20 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
13.3	CSPT1	20	1	0.05	Uterus	CR 54:4294
Unknown	Unknown	20	0	0	Brain	CR 50:5784
13.2	D20S4	15	2	0.13	Breast	GCC 2:191
Unknown	D20S119	26	3	0.12	Cervix	CR 56:197
13.2	D20S4	23	2	0.09	Cervix	CR 54:4481
Unknown	D20S25	25	0	0	Endocrine	CR 56:599
Unknown	D20S19	19	3	0.16	Esophageal	CR 54:2996
Unknown	D20S100	18	1	0.06	Head&Neck	CR 54:4756
Unknown	D20S100	21	2	0.1	Head&Neck	CR 54:4756
Unknown	D20S110	16	1	0.06	Head&Neck	CR 54:1152
Unknown	D20S119	11	1	0.09	Head&Neck	CR 54:1152
Unknown	D20S100	16	0	0	Kidney	PNAS 92:2854
Unknown	D20S100	26	0	0	Kidney	PNAS 92:2854
Unknown	Unknown	5	1	0.2	Liver	BJC 64:1083
13.2	D20S4	15	0	0	Liver	JJCR 81:108
13.2	D20S4	4	0	0	Liver	CCG 48:72
13.2	D20S4	10	1	0.1	Lung	PN 84:9252
13.2	D20S4	10	4	0.4	Lung	PN 86:5099
13.2	D20S4	2	2	1	Lung	PN 86:5099
13.2	D20S4	6	2	0.33	Lung	PN 86:5099
Unknown	D20S100	30	0	0	Melanoma	CR 56:589
Unknown	D20S19	33	0	0	Ovary	IJC 54:546
13.2	D20S4	19	3	0.16	Ovary	CR:53:2393
Unknown	D20S46	14	3	0.21	Ovary	BJC 69:429
Unknown	D20S54	14	1	0.07	Ovary	BJC 69:429
13.2	D20S4	8	0	0	Prostate	G 11:530
13.2	D20S4	11	0	0	Stomach	CR 48:2988
Unknown	D20S19	31	0	0	Testis	O 9:2245
Unknown	D20S26	25	1	0.04	Testis	GCC 13:249
13.2	D20S4	36	4	0.11	Testis	O 9:2245
13.3	CSPT1	20	1	0.05	Uterus	CR 54:4294
SUM		509	38	0.07		

Chromosome 21 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Refer
11.1	D21S52	13	1	0.08	Uterus	CR 51
Unknown	Unknown	14	0	0	Brain	CR 50
22.3	D21S113	5	0	0	Brain	CR 49
Unknown	BCEI	15	2	0.13	Breast	CR 53
Unknown	D21S1	21	1	0.05	Breast	GCC 2
Unknown	D21S112	29	4	0.14	Breast	CR 53
22.3	D21S113	26	4	0.15	Breast	CR 50
22.3	D21S113	3	0	0	Cervix	GCC 9
22.3	D21S113	19	2	0.11	Cervix	CR 54
Unknown	D21S212	26	2	0.08	Cervix	CR 56
Unknown	D21S265	23	0	0	Cervix	CR 56
Unknown	D21S267	14	1	0.07	Cervix	CR 56
Unknown	D21S1	15	0	0	Colon	CCG 4
Unknown	D21S156	16	0	0	Endocrine	CR 56
22.3	D21S113	9	2	0.22	Esophageal	CR 51
22.3	D21S113	30	11	0.37	Esophageal	GCC 1
22.3	D21S113	20	5	0.25	Esophageal	CR 54
Unknown	D21S262	18	0	0	Head&Neck	CR 54
Unknown	D21S262	17	3	0.18	Head&Neck	CR 54
Unknown	D21S59	19	5	0.26	Head&Neck	CR 54
22.3	D21S113	19	3	0.16	Kidney	CR 51
Unknown	D21S262	6	0	0	Kidney	PNAS
Unknown	D21S262	16	0	0	Kidney	PNAS
Unknown	D21S267-D21S265-D21S263	19	1	0.05	Kidney	PNAS
Unknown	D21S267-D21S265-D21S263	6	2	0.33	Kidney	PNAS
22.3	D21S113	15	1	0.07	Liver	CR 51
21.2-TER	D21S19	14	0	0	Liver	CCG
11.1	D21S52	4	1	0.25	Liver	JJCR
22.3	D21S113	28	5	0.18	Lung	CR 52
Unknown	D21S262	23	1	0.04	Melanoma	CR 56
22.3	D21S113	6	0	0	Ovary	O 5:2
22.3	D21S113	12	0	0	Ovary	CR 51
22.3	D21S113	25	2	0.08	Ovary	IJC 5
Unknown	D21S113-11	28	10	0.36	Ovary	CR 53
11.2	D21S120	12	4	0.33	Ovary	BJC 6
22.3	D21S167	13	7	0.54	Ovary	BJC 6
22.3-QTER	D21S171	13	3	0.23	Ovary	BJC 6
22.3	D21S113	3	0	0	Pancreas	CR 54
Unknown	D21S8-D21S17	10	0	0	Prostate	G 11:
Unknown	Unknown	6	2	0.33	Sarcoma	CGC 5
22.3	D21S113	15	1	0.07	Sarcoma	CR 52
22.3	D21S113	21	3	0.14	Testis	O 9:2
22.3	D21S113	6	1	0.17	Uterus	GCC 9
22.3	D21S167	20	0	0	Uterus	CR 54
11.1	D21S52	13	1	0.08	Uterus	CR 51

Chromosome 21 - q Arm

SUM	692	90	0.13

Chromosome 22 - q Arm

Band	Marker	Total	Cases w/LOH	LOH Freq.	Tumor Type	Reference
11.2-13.1	TOP1P2	15	1	0.07	Uterus	CR 54:4294
Unknown	BCR	2	0	0	Brain	CGC 53:271
Unknown	CRYB	7	1	0.14	Brain	CR 50:6783
Unknown	CYP2D	6	4	0.67	Brain	CR 53:2386
Unknown	CYP2D	6	6	1	Brain	CR 53:2386
11.2-12	D22S1	4	0	0	Brain	CR 50:6783
11.2-12	D22S1	7	2	0.29	Brain	CGC 53:271
11.1-11.2	D22S10	5	1	0.2	Brain	CGC 53:271
Unknown	D22S156	4	2	0.5	Brain	CR 53:2386
Unknown	D22S156	4	1	0.25	Brain	CR 53:2386
13.3	D22S171	2	0	0	Brain	CGC 66:117
11.2	D22S20	2	0	0	Brain	CGC 66:117
Unknown	D22S23	8	3	0.38	Brain	CR 50:6783
Unknown	D22S24	1	0	0	Brain	CR 50:6783
Unknown	D22S258	18	2	0.11	Brain	CR 54:1397
Unknown	D22S258	16	1	0.06	Brain	CR 54:1397
Unknown	D22S28	4	3	0.75	Brain	CR 50:6783
Unknown	D22S29	3	2	0.67	Brain	CR 50:6783
Unknown	D22S32	2	0	0	Brain	CGC 66:117
Unknown	D22S32	14	1	0.07	Brain	CR 49:6572
Unknown	D22S32	14	1	0.07	Brain	CR 8015784
13.1	D22S80	4	0	0	Brain	CGC 66:117
Unknown	D22S9	8	2	0.25	Brain	CGC 53:271
Unknown	D22S9	1	0	0	Brain	CGC 66:117
Unknown	IGLV	2	0	0	Brain	CGC 66:117
Unknown	IGLV	1	0	0	Brain	CR 50:6783
13	IL2RB	18	4	0.22	Brain	CR 54:1397
13	IL2RB	15	0	0	Brain	CR 54:1397
11.1-11.2	LAMBDALC	4	1	0.25	Brain	CGC 53:271
12.3	MB	5	0	0	Brain	CGC 66:117
12.3	MB	1	1	1	Brain	CGC 53:271
12.3-13.1	PDGFB	1	1	1	Brain	CGC 53:271
11	Unknown	26	10	0.38	Breast	JNCI 84:506
Unknown	D22S10	16	4	0.25	Breast	GCC 2:191
Unknown	D22S113	9	1	0.11	Breast	CR 50:7184
Unknown	D22S9	24	4	0.17	Breast	GCC 2:191
12.3	MB	42	8	0.19	Breast	CR 53:4956
11.1-11.2	D22S10	27	2	0.07	Cervix	CR 54:4481
Unknown	D22S113	8	1	0.12	Cervix	GCC 9:119
Unknown	D22S280	20	3	0.15	Cervix	CR 56:197
Unknown	D22S284	30	4	0.13	Cervix	CR 56:197
11.2-12	D22S1	11	1	0.09	Colon	N 331:273
11.2-12	D22S1	12	4	0.33	Colon	IJC 53:382
11.1-11.2	D22S10	12	0	0	Colon	S 241:961
11.1-11.2	D22S10	13	7	0.54	Colon	IJC 53:382
Unknown	D22S10	29	11	0.38	Colon	CR 50:7166

Chromosome 22 - q Arm

Unknown	D22S9	20	10	0.5	Colon	CR 50:7166
Unknown	D22S9	3	1	0.33	Colon	O 9:991
Unknown	D22S9	17	3	0.18	Colon	N 331:273
Unknown	IGLC	30	15	0.5	Colon	CR 50:7166
Unknown	IGLC	17	3	0.18	Colon	N 331:273
Unknown	IGLC	10	0	0	Colon	S 241:961
Unknown	IGLV	4	0	0	Colon	S 241:961
Unknown	IGLV	27	9	0.33	Colon	CR 50:7166
Unknown	IGLV	30	6	0.2	Colon	N 331:273
12.3-13.1	PDGFB	10	0	0	Colon	S 241:961
Unknown	SIS	4	1	0.25	Colon	N 331:273
Unknown	D22S264	16	0	0	Endocrine	GCC 13:9
Unknown	D22S351	19	1	0.05	Endocrine	CR 56:599
11.2-12	D22S1	21	2	0.1	Esophageal	CR 54:2996
Unknown	D22S32	13	1	0.08	Esophageal	GCC 10:177
Unknown	D22S79	18	3	0.17	Esophageal	CR 51:2113
Unknown	D22S283	25	2	0.08	Head&Neck	CR 54:4756
Unknown	D22S283	22	2	0.09	Head&Neck	CR 54:4756
13	IL2RB	24	7	0.29	Head&Neck	CR 54:1752
Unknown	D22S113	10	2	0.2	Kidney	CR 51:820
12	D22S268	39	1	0.03	Kidney	BJC 69:230
Unknown	D22S280-D22S282	22	0	0	Kidney	PNAS 92:2854
Unknown	D22S280-D22S282	6	0	0	Kidney	PNAS 92:2854
Unknown	D22S283	6	0	0	Kidney	PNAS 92:2854
Unknown	D22S283	16	0	0	Kidney	PNAS 92:2854
11.2-12	D22S1	10	0	0	Liver	JJCR 81:108
Unknown	D22S113	4	0	0	Liver	CR 51:89
Unknown	IGLC	28	9	0.32	Liver	JJCR 84:893
Unknown	IGLC	7	0	0	Liver	GCG 48:72
11.2-12	D22S1	7	2	0.29	Lung	CR 54:5643
11.2-12	D22S1	22	11	0.5	Lung	CR 54:5643
11.2-12	D22S1	3	2	0.67	Lung	CR 54:5643
Unknown	D22S113	16	3	0.19	Lung	CR 52:2478
Unknown	D22S283	35	2	0.06	Melanoma	CR 56:589
11.1-11.2	D22S10	13	3	0.23	Ovary	IJC 54:546
Unknown	D22S113	10	2	0.2	Ovary	CR 51:5118
Unknown	D22S156	10	3	0.3	Ovary	BJC 69:429
Unknown	D22S430-D22S282-D22S283-D22S274	32	23	0.72	Ovary	BJC 70:905
Unknown	D22S9	14	10	0.71	Ovary	CR 53:2393
Unknown	IL-2RB-CYP2D-D22S156	14	4	0.29	Ovary	BJC 72:1330
12.3-13.1	PDGFB	5	1	0.2	Ovary	CR 50:2724
Unknown	SIS	6	0	0	Ovary	CR 49:1220
11.2-13.1	TOP1P2	12	5	0.42	Ovary	BJC 69:429
Unknown	D22S113	4	0	0	Pancreas	CR 54:2761
Unknown	D22S156	26	20	0.77	Pediatric	GCC 15:10

Chromosome 22 - q Arm

Unknown	D22S257	20	10	0.5	Pediatric	GCC 15:10
Unknown	D22S258	23	18	0.78	Pediatric	GCC 15:10
Unknown	D22S264	26	9	0.35	Pediatric	GCC 15:10
Unknown	D22S273	21	14	0.67	Pediatric	GCC 15:10
Unknown	D22S273	26	16	0.62	Pediatric	GCC 15:10
Unknown	D22S274	14	10	0.71	Pediatric	GCC 15:10
Unknown	D22S275	17	13	0.76	Pediatric	GCC 15:10
Unknown	D22S280	25	17	0.68	Pediatric	GCC 15:10
Unknown	D22S281	20	12	0.6	Pediatric	GCC 15:10
Unknown	D22S283	29	18	0.62	Pediatric	GCC 15:10
Unknown	D22S301	20	14	0.7	Pediatric	GCC 15:10
Unknown	D22S303	21	12	0.57	Pediatric	GCC 15:10
Unknown	D22S315	26	18	0.69	Pediatric	GCC 15:10
Unknown	IGLV	10	0	0	Pediatric	CR 50:3279
12.3-13.1	PDGFB	7	1	0.14	Prostate	G 11:530
11.2-12	D22S1	21	8	0.38	Sarcoma	CR 52:2419
Unknown	D22S9	6	2	0.33	Sarcoma	CGC 53:45
11.2-12	D22S1	17	0	0	Stomach	CR 48:2980
Unknown	IGLC	7	2	0.29	Stomach	CR 52:3099
11.1-11.2	D22S10	26	6	0.23	Testis	O 9:2245
12.3-13.1	PDGFB	3	0	0	Testis	CCG 52:72
12.3-13.1	PDGFB	2	0	0	Testis	CCG 52:72
12.3-13.1	PDGFB	1	0	0	Testis	CCG 52:72
Unknown	D22S113	16	3	0.19	Uterus	GCC 9:119
11.2-13.1	TOPIP2	15	1	0.07	Uterus	CR 54:4294
SUM		1594	472	0.3		

Chromosome	Arm	LOH Freq.
1	p	0.26
1	q	0.15
2	p	0.15
2	q	0.12
3	p	0.17
3	q	0.18
4	p	0.11
4	q	0.22
5	p	0.19
5	q	0.27
6	p	0.24
6	q	0.25
7	p	0.12
7	q	0.22
8	p	0.33
8	q	0.14
9	p	0.38
9	q	0.47
10	p	0.18
10	q	0.23
11	p	0.23
11	q	0.26
12	p	0.15
12	q	0.13
13	q	0.29
14	p	0.08
14	q	0.22
15	p	0.11
15	q	0.17
16	p	0.17
16	q	0.36
17	p	0.44
17	q	0.31
18	p	0.12
18	q	0.29
19	p	0.13
19	q	0.3
20	p	0.11
20	q	0.07
21	q	0.13
22	q	0.3

Fig. 5

1) Cyclins

Validation: Deletion of CDC23(Anaphase Promoting), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
9	CDC-25A	1	3p21	U54831
10	CDC-25C	1	5q31	M34065
524	Wee1	3	1p15.3-p15.1	X62048
1043	CDC16Hs	2	13	U18291
1278	Cyclin D1	4	11q13	M73554
1280	Cyclin D3	2	6p21	M90814
1298	Cyclin H Assembly Factor	1	4	X87843
1445	Cyclin-Dependent Protein Kinase	2	12	U79269
1450	RAN binding protein 1	1	22	D38076
1523	14-3-3 PROTEIN TAU	1	10	X56468

1) Cyclin dependent kinases/phosphatases

Validation: Deletion of CDC28 (Cyclin Dependent Protein Kinase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1051	CDC28 protein kinase 1	2	17	X54941
1052	CDC28 protein kinase 2	1	9	X54942
1111	Protein phosphatase 1, catalytic subunit, alpha isoform	4	11	M63960
1388	M-PHASE INDUCER PHOSPHATASE 2	1	20	M81934
1401	M-phase phosphoprotein, mpp6	5	7	X98263

1) Cell Division Structural Proteins

Validation: Deletion of CBF2 (Kinetochore Protein), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
20	MCM7 (Minichromosome Maintainence	3	7q21.3-q22.1	U20980
1246	Chromatin assembly factor-I p60 subunit	2	21	U20980
1273	Chromosome segregation gene homolog CAS	1	20	U33286
1347	High-mobility group (nonhistone chromosomal) protein 1	5	13q12	D63874
1487	Chromatin structural protein homolog (SUPT5H)	3	7	Y12790
1607	Centromere protein B (80kD)	1	20p13	X05299

2) Uniporters

Validation: Deletion of SAT2(Osmotolerance), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1253	ATPase, Ca++ transporting, plasma membrane 2	5	3p26-p25	X63575
1255	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	4	12p13-qter	X03559
1286	Putative Chloride Channel	1	13q14.3-q21.1	X83378
1337	Copper Transport Protein HAH1	1	5	U70660
1407	Nuclear chloride ion channel protein (NCC27)	4	20	U93205
1463	Sodium channel, voltage-gated, type I, beta polypeptide	1	19q13.1	L16242
1505	Transient receptor potential channel 1	1	3	X89066
1521	Voltage-dependent anion channel 2	4		L06328

2) Antiporters

Validation: Proven essential in mammalian cells by tritium suicide selection experiments.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1471	Solute carrier family 9 (sodium/hydrogen exchanger)	1	1p36.1-p35	M81768
1250	ATPase, Na+/K+ transporting, beta 1 polypeptide	1	1q22-q25	X03747
1251	ATPase, Na+/K+ transporting, beta 2 polypeptide	2	17p	M81181
1605	Solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)	2	7q35-q36	U62531

3) Acyltransferase

Validation: Essential for metabolic processes such as biosynthetic reactions and energy metabolism. The S. cerevisiae histone acetyltransferase PAT1 and the N-alpha acetyltransferase which acetylates the N-termini of proteins are essential for growth.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1227	Acetyl-Coenzyme A acyltransferase (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	2	3p23-p22	X12966
1387	Lysophosphatidic acid acyltransferase-alpha	7	6	U56417

3) Amino Acid Biogenesis

Validation: Deletion of PRO1(Glutamate 5-Kinase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1330	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	1	10q24.1-q25.1	M37400
1331	Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	2	16q21	M22632
1447	Pyrroline-5-carboxylate synthetase (glutamate gamma-semialdehyde synthetase)	1	10q24.3	X94453

3) Amino Acid Transport

Validation: There are ten essential amino acids in man, which must be transported across the plasma membrane for use in protein synthesis.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1581	Solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1	2	2p16.3	L11696

3) Addition, removal, or modification of phosphate groups

Validation: Deletion of CMD1(Calmodulin), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1269	Calcineurin A catalytic subunit	2	8	S46622
1270	Calcineurin B	1	10q21-q22	M30773
1351	CALRETICULIN PRECURSOR	1	10q21-q22	M84739
1432	SERINE/THREONINE PROTEIN PHOSPHATASE 2B CATALYTIC SUBUNIT, BETA ISOFORM	2	10	M29551
1476	Snk interacting protein 2-28	1		U83236

3) GDP Dissociation Inhibitors

Validation: Deletion of GDI1(GDP dissociation Factor), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1448	RAB GDP DISSOCIATION INHIBITOR ALPHA	2	14q23-q24	D13988

3) Lactate Transport

Validation: Genes required to maintain organic compounds at levels required for cell growth or survival.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1583	Solute carrier family 16 (monocarboxylic acid transporters), member 1	2	1p13.2-p12	L31801

3) Polyamine Biosynthesis

Validation: Inhibition of polyamine biosynthesis has antiproliferative effects as demonstrated by inhibitors of polyamine metabolism.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1587	Ornithine decarboxylase 1	2	2p25	M16650

3) Protein Glycosylation

Validation: Deletion of DPM1(Dolichol-phosphate mannosyltransferase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1328	Glutamine-fructose-6-phosphate transaminase	1	2p13	M90516
1339	Heparan Heparan Heparan Heparan N-deacetylase/N-sulfotransferase-2	2	10	U36601
1434	UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase	3	18	U41514

3) Protein Kinase C

Validation: Deletion of PKC1(Protein Kinase C), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1440	Protein kinase C, beta 1	4	16p11.2	X06318
1443	Protein kinase C-theta	1	10p15	L01087
1444	Protein kinase C substrate 80K-H	1	7	J03075

3) Protein Post-modification

Validation: Deletion of BET2(Geranylgeranyltransferase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1081	geranylgeranyl transferase type II beta-subunit	2	1	X98001

3) Sugar Biosynthesis and Processing

Validation: Deletion of PGI1(Glucose-6-phosphate Isomerase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
14	PIP 5 Kinase beta	2	9q13	X92493
1229	Aconitase 2, mitochondrial	1	22q11.21-q13.3	U80040
1249	ATP SYNTHASE ALPHA CHAIN, MITOCHONDRIAL PRECURSOR	2	18	D14710
1257	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	3	18	X60221
1258	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein)	5	21q22.1-q22.2	X83218
1302	Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)	5	11	AF001437
1303	Dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex)	5	7q31-q32	J03490
1346	Hexokinase 1	3	10q22	M75126
1366	Isocitrate dehydrogenase 2 (NADP+), mitochondrial	2	15q26.1	X69433
1395	NADH dehydrogenase	1	2p16	X81900
1421	NADH:ubiquinone oxidoreductase subunit B13	4	18p11.31-p11.2	U53468
1422	NADH dehydrogenase-ubiquinone Fe-S protein 8, 23 kDa subunit precursor (NDUFS8)	1	18p11.31-p11.2	U65579
1424	NADH-UBIQUINONE OXIDOREDUCTASE 75 KD SUBUNIT PRECURSOR	3	2	X61100
1427	Pyruvate dehydrogenase (lipoamide) beta	9	3p13-q23	M34479
1430	Phosphofructokinase	1	21q22.3	M10036
1451	UBIQUINOL-CYTOCHROME C REDUCTASE COMPLEX 11 KD PROTEIN PRECURSOR	3	1,3	M36647
1464	Succinate dehydrogenase, iron sulphur (Ip) subunit	3	1p22.1-qter	D10245
1465	Succinate dehydrogenase 2, flavoprotein (Fp) subunit	10	5p15	D30648
1576	Pyruvate kinase, liver	2	1q21	D10326
1577	Oxoglutarate dehydrogenase (lipoamide)	6	7p14-p13	D10523
1579	Acyl-Coenzyme A dehydrogenase, very	3	17p11.2-p11.13	D43682

	long chain			
1584	Dihydrolipoamide S-succinyltransferase	5	14q24.3	L37418
1588	Acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	1	1p31	M16827
1590	Pyruvate kinase, muscle	4	15q22	M23725
1596	Phosphoglucomutase 1	5	1p31	M83088
1603	Phosphofructokinase, muscle	4	12q13.3	U24183
1611	Enolase 3, (beta, muscle)	1	17pter-p12	X16504

3) Sugar Transport

Validation: Genes required to maintain organic compounds at levels required for cell growth or survival.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1594	Solute carrier family 2 (facilitated glucose transporter), member 5	3	1p31	M55531
1598	Solute carrier family 5 (sodium/glucose cotransporter), member 2	1	16	M95549

4) Protein Degradation

Validation: Deletion of CDC48(Ubiquitin proteolysis), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1027	26S PROTEASE REGULATORY SUBUNIT 4	3	14	L02426
1037	CALPAIN 1, LARGE	1	11	X04366
1098	Human mRNA for KIAA0123 gene, partial cds	6	9,19	D50913
1114	Proteasome (prosome, macropain) subunit, beta type, 6	7	9,19	D29012
1115	Human mRNA for proteasome subunit z, complete cds	4	9	D38048
1116	PROTEASOME COMPONENT C13 PRECURSOR	2	9	U17496
1117	Human mRNA for proteasome subunit HsC7-I, complete cds	6	1	D26599
1118	Human mRNA for proteasome subunit p112, complete cds	2	2	D44466
1119	Human mRNA for proteasome subunit p27, complete cds	1	2	AB003177
1289	ATP-DEPENDENT CLP PROTEASE PROTEOLYTIC SUBUNIT	2	19	Z50853

4) Prot in Folding

Validation: Deletion of HSP10(Chaperonin), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence

1287	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE, MITOCHONDRIAL PRECURSOR	1	10	M80254
1305	DNAJ PROTEIN HOMOLOG 2	1	9,2	D13388
1358	DNAJ PROTEIN HOMOLOG HSJ1	2	9,2	X63368

4) Ribosomal Subunit

Validation: Deletion of GRC5(Ribosome), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1127	H.sapiens mRNA for ribosomal protein L11	3	9,2	X79234
1128	Ribosomal protein L17	2	17,4	X52839
1130	60S RIBOSOMAL PROTEIN L18A	5	3	X80822
1131	Ribosomal protein L19	1	17q11	X63527
1133	60S RIBOSOMAL PROTEIN L23A	2	17,18	U43701
1135	Human ribosomal protein L27a mRNA, complete cds	3	6,11	U14968
1136	Human ribosomal protein L28 mRNA, complete cds	11	19	U14969
1137	Ribosomal protein L32	4	20	X03342
1138	Human ribosomal protein L35 mRNA, complete cds	3	20	U12465
1139	Ribosomal protein L35a	1	3q29-qter	X52966
1140	Human mRNA for ribosomal protein L39, complete cds	2	3q29-qter	U57846
1141	Ribosomal protein L4	4	3,6	L20868
1142	Ribosomal protein L6	1	12	X69391
1143	Ribosomal protein L7	1	12	L16558
5	Ribosomal protein L7A	1	19q33-q34	M36072
1144	Ribosomal protein L8	5	12	Z28407
1145	Ribosomal protein L9	2	12	U09953
1146	Ribosomal protein, large, P1	5	15,22	M17886
1147	Human ribosomal protein S10 mRNA, complete cds	1	20	U14972
1148	Ribosomal protein S11	1	19q	X06617
1149	40S RIBOSOMAL PROTEIN S15	2	19q	J02984
1150	40S RIBOSOMAL PROTEIN S15A	2	19q	X84407
1151	Ribosomal protein S16	5	19	M60854
1152	Ribosomal protein S17	5	11pter-p13	M13932
1154	40S RIBOSOMAL PROTEIN S23	2	5	D14530
1155	Ribosomal protein S25	2	11q23.3	M64716
1157	Ribosomal protein S28	2	19	U58682
1158	40S RIBOSOMAL PROTEIN S29	1	19	L31610
1159	Ribosomal protein S5	2	19	U14970
1160	40S RIBOSOMAL PROTEIN S7	3	19	M77233
1161	Ribosomal protein S9	3	19	U14971
1223	Ribosomal protein L7a	6	9q34	X52138

4) T-Complex

Validation: Deletion of CCT2(T-Complex), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1489	T-COMPLEX PROTEIN 1, ALPHA SUBUNIT	1	6	S70154

1490	T-COMPLEX PROTEIN 1, EPSILON SUBUNIT	3	5	D43950
1491	T-COMPLEX PROTEIN 1, GAMMA SUBUNIT	2	1	X74801

4) Translation Elongation

Validation: Deletion of CDC33(eIF4e), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1063	Eukaryotic translation elongation factor 1 delta	3	7	Z21507
1073	Eukaryotic translation initiation factor 4A (eIF-4A) isoform 2	1	18p11.2	D30655
1095	Human mRNA for KIAA0031 gene, complete cds	3	17,2	D21163
1099	Human mRNA for KIAA0219 gene, partial cds	3	12	D86973

4) Translation Factor

Validation: Deletion of CDC33(eIF4e), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1049	PEPTIDE CHAIN RELEASE FACTOR SUBUNIT 1	2	12	X81625

4) Translation Initiation Factors

Validation: Deletion of CDC33(eIF4e), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1068	Human translation initiation factor eIF-3 p110 subunit gene	1	16	U46025
1069	EUKARYOTIC INITIATION FACTOR 4A-LIKE NUK-34	1	17	D21853
1070	Eukaryotic translation initiation factor 4C (eIF-4C)	3	1,X	L18960
1072	Eukaryotic translation initiation factor 2A	2	14	J02645
1074	Eukaryotic translation initiation factor 4E	3	14	M15353
1312	Translation initiation factor 3 (eIF-3) p36 subunit	1	12	U39067

4) tRNA Synthetases

Validation: Deletion of ALA1(Alanyl-tRNA synthetase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1031	Alanyl-tRNA synthetase	2	16q22	D32050
1040	Cysteinyl-tRNA synthetase	1	11p15.5	L06845
1079	Glycyl-tRNA synthetase	2	7p15	U09510
1090	Isoleucine-tRNA synthetase	2	9q21	D28473
1102	ASPARAGINE SYNTHETASE	3		M27396
1121	Arginyl-tRNA synthetase	3	5pter-q11	S80343
1198	Threonyl-tRNA synthetase	1	5p13-cen	M63180
1218	VALYL-TRNA SYNTHETASE	4	9	X59303
1221	TRYPTOPHANYL-TRNA SYNTHETASE	1	14	M61715

4) Ubiquitin and Ubiquitin Associated

Validation: Deletion of UFD1(Ubiquitin Fusion), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1309	Ubiquitin carrier protein (E2-EPF)	2	17	M91670
1315	Cyclin-selective ubiquitin carrier protein	2	17	U73379
1362	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 3	2	14	D80012
1363	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE T	1	12	X91349
1420	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 14	4	13	M68864
1431	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE ISOZYME L1	2	4	X04741
1511	Ubiquitin A-52 residue ribosomal protein fusion product 1	1	19p13.1-p12	S79522
1514	Ubiquitin-conjugating enzyme E2I	6	16p13.3	U45328
1515	Ubiquitin fusion-degradation protein (UFD1L)	4	18	U64444

5) DNA Helicases

Validation: Deletion of DNA2(DNA Helicase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1050	Human CHL1 potential helicase (CHLR1), complete cds	3	18	U33833
1057	ATP-DEPENDENT DNA HELICASE II, 86 KD SUBUNIT	1	2	M30938
1123	RecQ protein-like (DNA helicase Q1-like)	2	12p12-p11	L36140
1397	218kD Mi-2 protein	1	12	X86691

5) DNA Polym ras

Validation: Deletion of POL2(DNA pol epsilon), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1059	Human DNA polymerase delta small subunit mRNA, complete cds	3	12	U21090
1105	DNA polymerase alpha subunit	1	X,11	L24559

5) DNA Replication

Validation: Deletion of CDC45(Chromosomal DNA Replication), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1048	DNA REPLICATION LICENSING FACTOR CDC47 HOMOLOG	1	4	D55716
1094	Human mRNA for KIAA0030 gene, partial cds	2	3	X67334
1124	Replication factor C (activator 1) 1 (145kD)	2	4p14-p13	L14922
1208	DNA topoisomerase I	2	20q12-q13.1	J03250
22	Topoisomerase II	2	17q21-q22	J04088
1222	Minichromosome maintenance deficient (S. cerevisiae) 3	1	17q21-q22	D38073
1461	Replication protein A2 (32kD)	2	1p35	J05249

5) Histone

Validation: Deletion of CSE4(Similar Histone H3), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1335	Histone H1(0)	3	22	X03473
1336	Histone H1x	3	22	D64142
1341	HISTONE H1D	5	6	X57129
1342	HISTONE H2A.1	4	6	U90551
1343	Histone H2A.2	1	6	L19779
1344	Histone H2B.1	1	1	M60756
1345	H4 histone	1	1	X60486

5) Polyadenylation and 3' Cleavage

Validation: Deletion of FIP1(Polyadenylation Factor), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence

1053	Human cleavage and polyadenylation specificity factor mRNA, complete cds	1	11	U37012
1349	HNRNP METHYLTRANSFERASE	4	14	D66904
1426	Poly(A)-binding protein-like 1	2	14	Y00345

5) Purine/Pyrimidine Biosynthesis

Validation: Deletion of CDC8(Thymidylate Kinase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1235	ADENYLOSUCCINATE LYASE	1	1	X65867
1268	CAD PROTEIN	1	2	D78586
1293	CTP synthetase	2	1p34.1	X52142
1326	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	4	21q22.1	X54199
1437	Phosphoribosyl pyrophosphate amidotransferase	2	4q12	U00238
1510	Thymidylate synthase	2	18p11.32	X02308
1517	Uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase)	2	3q13	J03626
1518	Uridine Phosphorylase	1	7	X90858

5) Ribonucleotide Reductase

Validation: Deletion of RNR1(Ribonucleotide Reductase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1452	RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE M1 CHAIN	4	11	X59543

5) RNA Helicase

Validation: Deletion of BRR2(RNA Helicase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1100	Human mRNA for KIAA0224 gene, complete cds	4	16	D86977
1163	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 9 (RNA helicase A)	1	1	L13848
1484	PUTATIVE ATP-DEPENDENT RNA HELICASE STE13	3	19	U90426

5) RNA Polymerase II Components

Validation: Deletion of RPA135(RNA pol Subunit), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1026	Homo sapiens (clone mf.18) RNA polymerase II mRNA, complete cds	3	19	L37127
1088	Human RNA polymerase II subunit (hsRPB10) mRNA, complete cds	7	19	U37690
1109	RNA polymerase II, polypeptide C (33kD)	3	16q13-qq21	J05448
1110	Polymerase (RNA) II (DNA directed) polypeptide A (220kD)	1	17p13.1	X63564
1165	DNA-DIRECTED RNA POLYMERASE II 23 KD POLYPEPTIDE	9	17p13.1	J04965
1360	RNA polymerase II subunit hsRPB7	1	11	U20659

5) RNA Polymerase III

Validation: Deletion of RPA135(RNA pol Subunit), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1170	Human RNA polymerase III subunit (RPC62) mRNA, complete cds	1	11	U93867

5) RNA Splicing/Processing

Validation: Deletion of CUS1(U2 snRNP protein), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1171	Human spliceosome associated protein (SAP 145) mRNA, complete cds	1	2	U41371
1172	Human splicesomal protein (SAP 61) mRNA, complete cds	3	2	U08815
1176	H.sapiens mRNA for splicing factor SF3a120	1	22	X85237
1177	Splicing factor, arginine/serine-rich 2	2	4,17	M90104
1181	Human splicing factor SRp30c mRNA, complete cds	1	6	U30825
1183	PRE-MRNA SPLICING FACTOR SRP75	2	1	L14076
1216	SPLICING FACTOR U2AF 65 KD SUBUNIT	1	1	X64044
1224	Human (clone E5.1) RNA-binding protein mRNA, complete cds	4	1	L37368
1322	Fibrillarin	1	1	X56597
1354	Heterogeneous nuclear ribonucleoprotein K	1	9q21.32-q21.33	S74678

1455	U1 SMALL NUCLEAR RIBONUCLEOPROTEIN A	3	9q21.32-q21.33	X06347
1460	U1 small nuclear RNP-specific C	2	15	X12517
1473	SnRNP core protein Sm D3	2	22	U15009
1474	SnRNP core protein Sm D2	5	22	U15008
1477	U1 snRNP 70K protein	3	19q13.3	M22636
1478	Small nuclear ribonucleoprotein polypeptides B and B1	3	20	J04564
1480	Small nuclear ribonucleoprotein polypeptide N	5	15q12	U41303

5) TATA-Binding Proteins

Validation: Deletion of TAF145(TAFII Complex), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1193	H.sapiens mRNA for transcription factor TFIID subunit TAFII28	1	6	X83928
1196	Human TFIID subunit TAFII55 (TAFII55) mRNA, complete cds	1	5	U18062
1199	TATA box binding protein	2	6q27	M55654
1361	TBP-associated factor (hTAFII130)	1	20	U75308

5) Transcription Elongation Factors

Validation: Deletion of RPO21(RNA pol Subunit), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1077	TRANSCRIPTION ELONGATION FACTOR S-II	4	8	M81601
4	TRANSCRIPTION ELONGATION FACTOR B3	5	5q31	L34587
32	Elongin TCEB1	3	1p36.1	L47345

5) Transcription Factors

Validation: Deletion of BBP1(BFR1p binding), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
33	SUPT6H	3	17q11.2	U46691
1202	Human TFIIA gamma subunit mRNA, complete cds	1	15	U14193
1205	General transcription factor TFIIE beta subunit, 34 kD	1	8p21-p12	X63469
1206	TRANSCRIPTION INITIATION FACTOR IIF, BETA SUBUNIT	1	8p21-p12	X16901
1247	CYCLIC-AMP-DEPENDENT TRANSCRIPTION FACTOR ATF-1	1	19p13.3	X55544
1248	CAMP-dependent transcription factor	3	2	M86842

	ATF-4 (CREB2)			
1274	Transcription Factor (CBFB)	1	2	L20298
1292	CRM1 protein	3	2	Y08614
1368	Transcription Factor IL-4 Stat	1	21q21-q22.1	U16031
1373	SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1-ALPHA/BETA	1	21q21-q22.1	M97935
1411	Nuclear Factor I-B2 (NFIB2)	1	19	U85193
1483	Transcription Factor Stat5b	1	17	U48730
1496	Transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)	2	15q21	M83233
1497	Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	8	19p13.3	M31523
1498	Transcription factor 6-like 1 (mitochondrial transcription factor 1-like)	1	7p	M62810
1500	TRANSCRIPTION FACTOR P65	3	11	L19067
1501	Transcription factor COUP 2 (a.k.a. ARP1)	2	15q26.1-q26.2	X91504

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6) Clathrin

Validation: Deletion of RET1(Alpha-Cop), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1242	CLATHRIN COAT ASSEMBLY PROTEIN AP47	2	8	D38293
1243	CLATHRIN COAT ASSEMBLY PROTEIN AP50	6	3	U36188
1282	cell surface protein	5	22	X83545
1290	Clathrin, light polypeptide (Lcb)	1	4q2-q3	M20470
1291	Clathrin heavy chain	4	17q11-qter	U41763

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6) Cytoskeleton

Validation: Deletion of MHP1(Microtubule Interacting), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1	Actin, gamma Subunit	8	17p11-qter	X04098
	Sh3p17(Myosin IC Heavy Chain)	1	21	U61166
1032	Actin depolymerizing factor [human, fetal brain, mRNA, 1452 nt]	4	20	S65738
1038	Capping protein (actin filament), gelsolin-like	3	2cen-q24	M94345
1039	Human capping protein alpha mRNA, partial cds	2	7	U03851
1056	Desmin	1	2q35	J03191
1080	Gelsolin (amyloidosis, Finnish type)	1	9q34	X04412
1092	Keratin 19	5		Y00503
1093	KERATIN, TYPE II CYTOSKELETAL 6D	13	5,12	J00269
1267	BETA-CENTRACTIN	1	2	X82207
1284	Cofilin 1 (non-muscle)	5	11q13	X95404
1383	LAMIN A	1	20	M13451
1385	Lamin B receptor	1	1q42.1	L25931

1386	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM	1	12,17	M22920
1404	MYOSIN HEAVY CHAIN 95F	1	4p16.3	U90236
1405	MYOSIN HEAVY CHAIN IB	1	13	D63476
1406	Myosin-IC	1	13	U14391
1486	SUPPRESSOR OF TUBULIN STU2	1	11	X92474
1495	MICROTUBULE-ASSOCIATED PROTEIN TAU	1	17	J03778
1507	Tubulin, gamma polypeptide	1	17	M61764
1508	TUBULIN ALPHA-4 CHAIN	1	17	X06956
1520	Myosin VIIA (USH1B)	2	17	U39226

6) ER Protein

Validation: Deletion of BET1(v-SNARE), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1272	Calnexin	1	5q35	M94859
1317	ER LUMEN PROTEIN RETAINING RECEPTOR 2	1	19	M88458
1614	Ribophorin I	4	3q	Y00281
1615	Ribophorin II	1	20q12-q13.1	Y00282

6) Integrin

Validation: Deletion of MYO2(Myosin Heavy Chain), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1378	Integrin alpha-3 subunit	1	5q23-q31	M59911

6) Karyopherin

Validation: Deletion of KAP121(Karyopherin), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1091	karyopherin alpha 3	3	13	D89618
1214	transportin (TRN)	1	13	U70322

6) Lysos mal Proteins

Validation: Essential for sequestering and degrading aged or defective organelles and polymers that can interfere with cell survival, proliferation as seen by human diseases such as Tay-Sachs disease.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1265	ATPase, H+ transporting, lysosomal (vacuolar proton pump) 31kD	2	22pter-q11.2	X76228

6) MITOCHONDRIAL IMPORT

Validation: Genes required to maintain inorganic ions at levels compatible with cell growth or survival.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1578	MITOCHONDRIAL IMPORT RECEPTOR SUBUNIT TOM20	8	1	D13641

6) Nuclear Pore Complex

Validation: Deletion of GSP1(Nuclear Pore Trafficking), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
28	Nuclear Pore Complex NUP214	3	9	D14689
29	Nucleoporin 98	3	11p15	U41815
1266	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN C	4	20	L38696
1350	Heterogeneous nuclear ribonucleoprotein A1	4	12q13.1	X79536
1355	Nuclear pore complex protein hnup153	3	6	Z25535
1425	NUCLEAR PORE GLYCOPROTEIN P62	1	11	X58521
1449	Export protein Rae1	5	20	U84720
1454	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEINS C1/C2	3	12	M29063
1524	140 KD NUCLEOLAR PHOSPHOPROTEIN	5	10	D21262

6) Protein Transport

Validation: Deletion of BET3(v-SNARE associated), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
8	Integral Transmembrane Protein	3	11q23-24	L38961
1467	Sec23A isoform	2	14	X97064
1608	Signal recognition particle receptor ('docking protein')	8	11q23-q24	X06272
1613	TIM17 preprotein translocase	2	1	X97544

6) Syntaxin

Validation: Deletion of SED5(Syntaxin), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1186	syntaxin 1A	1	21q22.1	L37792
1188	syntaxin 3	1	11	U32315
1189	Syntaxin 5A	2	11	U26648
1190	syntaxin 7	1	6	U77942

6) Vacuolar Protein

Validation: Deletion of PPA1(Vacuolar H-ATPase), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1261	Vacuolar H+ ATPase proton channel subunit	2	6	M62762

6) Vesicle Proteins

Validation: Deletion of SAR1(COP II), a S. cerevisiae gene in the same biochemical family, is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1025	Human (chromosome 3p25) membrane protein mRNA	3	3,18	L09260
24	COATOMER BETA SUBUNIT	1	3	X70476
1055	COATOMER DELTA SUBUNIT	8	11	X81198
1082	Human GP36b glycoprotein mRNA, complete cds	3	5	U10362
1173	SEC14 (S. cerevisiae)-like	7	17q25.1-q25.2	D67029
1174	Human homologue of yeast sec7 mRNA, complete cds	2	17q25.1-q25.2	M85169
1184	Human chromosome 17q21 mRNA clone LF113	1	17	U18009
1217	H.sapiens mRNA for vacuolar-type H(+)-ATPase 115 kDa subunit	2	17	Z71460

99) Direct Essential Yeast Homolog

Validation: Deletion of the S. cerevisiae homologue of this gene is lethal.

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1238	Aldolase A	2	16q22-q24	M11560
1239	Aldolase B, fructose-bisphosphate	2	9q22	X02747
1241	S-adenosylmethionine decarboxylase 1	1	6q21-q22	M21154
1271	Calmodulin 1 (phosphorylase kinase, delta)	1	14q24-q31	D45887
1300	DED81	1	18	U79254

1301	Deoxyhypusine synthase	3	19p13.11-p13.12	L39068
1306	Dolichol monophosphate mannose synthase (DPM1)	2	20	AF007875
1318	ESS1 PROTEIN	1	19	U49070
1332	Glucose phosphate isomerase	1	19q13.1	K03515
1333	Guanylate kinase (GUK1)	3	19q13.1	L76200
1359	Heat shock 60 kD protein 1 (chaperonin)	1	9	M34664
1367	PERIODIC TRYPTOPHAN PROTEIN 1	1	12	L07758
1372	IPP isomerase	1	10	X17025
1396	N-acetylglucosaminyltransferase I	4	5q31.2-q31.3	M55621
1399	Mannose phosphate isomerase	3	15q22-qter	X76057
1414	Nip1	1	5	U15172
1415	GLYCYLPEPTIDE N-TETRADECANOYLTRANSFERASE	2	17	M86707
1433	PHOSPHATIDYLINOSITOL 4-KINASE ALPHA	10	17	L36151
1446	PERIODIC TRYPTOPHAN PROTEIN 2	2	8	U53346
1519	Uridine diphosphoglucose pyrophosphorylase	1	2	U27460

Fig. 6

Target Variances by Field Table for Conditionally Essential Genes

Conditionally Essential Biosynthetic Enzymes

Validation: Conditionally Essential

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1536	5-methyltetrahydrofolate-homocysteine methyltransferase	3		U75743
1539	Glutamate-ammonia ligase (glutamine synthase)	5	1q31	X59834

Proteins that Repair Radiation Induced DNA Damage

Validation: Conditionally Essential

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1541	Fanconi anemia complementation group C	1	9q22.3	X66894

Proteins of DNA Repair

Validation: Conditionally Essential

ID	Name	Variances Identified	Chromosome	Genbank Sequence
1528	DNA excision repair protein ERCC5	4	13q33	D16305
1530	HHR23A protein	3	9	D21235
1532	DNA EXCISION REPAIR PROTEIN ERCC-1	2	19q13.2-q13.3	M13194
1533	DNA repair helicase ERCC3	1	2q21	M31899
1537	URACIL-DNA GLYCOSYLASE 1 PRECURSOR	2	8	X15653
1526	Damage-specific DNA binding protein 1 (127 kD)	2	11, 15	AJ002955

Proteins that repair chemically induced DNA damag

Validation: Conditionally Essential

ID	Name	Variances	Chromosome	Genbank
1534	O-6-methylguanine-DNA methyltransferase	4	10q26	M60761

Fig. 7

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1.01	472	CGGCCATGTA[C/T]GTGGCCATCC	71 (36)	1 (1)	Silent
.02	250	ACGAGGCCCA[G/A]AGCAAGCGTG	71 (36)	1 (1)	Silent
.03	1003	CGGGCATTGC[C/T]GACAGGATGC	66 (35)	6 (5)	Silent
.04	801	ACGAGCTGCC[C/T]GATGGCCAGG	71 (36)	1 (1)	Silent
.05	1201	AATGCTTCTA[A/G]ACGGACTCAG	71 (36)	1 (1)	Silent
.06	991	CCACCATGTA[C/T]CCGGGCATTG	17 (17)	56 (35)	Silent
.07	1099	TGTGGATCGG[T/C]GGCTCCATCC	71 (36)	1 (1)	Silent
.08	499	GTGCTGTCCCT[C/G]TACGCCTCT	65 (65)	7 (7)	Silent
4.01	2168	CCGCCAGTAG[C/T]ATCAGCTTTA	61(34)	11(9)	3'UT
.02	388	TGGAAAGCCA[C/T]GGGGAGCCGA	62(29)	10(7)	Thr->Met
.03	491	AGAGGAGAGA[T/C]GAGAGAAAGA	68(36)	4(4)	Silent
.04	1171	AAAACTAATT[T/C]GGATAGAAAG	68(36)	4(4)	Leu->Ala
.05	336	TCGGGATGCC[C/T]TGCAGAAGGA	71(36)	1(1)	Silent
5.01	421	ACGTCCCAAC[G/A]AAGAGACCAC	66(36)	6(6)	Silent
8.01	1570	CTCCGTCCA[T/C]TGTACTATCTG	70(36)	2(2)	Silent
.02	778	TCCACGTCCT[C/G]GTGCTGATGC	71(36)	1(1)	Silent
.03	158	GGACACACTT[T/C]TGAAGCTTCT	71(36)	1(1)	Silent
9.01	1929	CCATGCACCA[C/A]GAGGACTTTA	71(36)	1(1)	His->Gln
10.01	1099	AACCGTGTCAGGGAAACACCA	69(36)	3(3)	Gly->Arg
14.01	911	CAATTCAATC[G/A]CCGCCCTAAA	69(36)	3(3)	Arg->His
.02	1174	CAAACAGTAA[G/A]TGAAAATGGT	71(36)	1(1)	
20.01	1627	CCCAGCACAT[C/T]ACCTATGTGC	44(30)	28(21)	Silent
.02	2041	GCCGAAGTGT[C/G]CGGTTCTCTG	71(36)	1(1)	Asp->Glu
.03	1393	cagccatcca[c/t]gaggtcatgg	71(36)	1(1)	Silent
22.01	4008	CAACAAAAAC[A/C]AAATTCACAA	71(36)	1(1)	Silent
.02	4446	AGCCATCCAC[T/G]TCTGATGATT	71(36)	1(1)	Silent
24.01	1101	GCCACTGGCA[G/A]TAAAGGATAT	71(36)	1(1)	Val->Ile
28.01	5009	TGCCACGCCC[G/C]TGTTTGGGCA	70(36)	2(2)	Val->Leu
.02	2023	AGAAATCACC[C/T]AGGATAACCC	71(36)	1(1)	Silent
.03	2041	CCCCTCCAGC[G/A]GCAAAGCCAG	71(36)	1(1)	Silent
29.01	1768	CCCTGCCACT[A/C]GAGTCCGGCC	67(36)	5(5)	Silent
.02	2781	AGGAGCATCC[G/A]TCTAAAACTA	70(36)	2(2)	Silent
.03		2 bp deletion			3'UT
32.01	1171	AAAACTAATT[T/C]GGATAGAAAG	70(36)	2(2)	Leu->Ala
.02	388	TGGAAAGCCA[C/T]GGGGAGCCGA	59(33)	13(10)	Pro->Met
.03	2168	CCGCCAGTAGCATCAGCTTTA	60(34)	12(10)	Silent
33.01	2397	GGCTAGATGG[T/C]CTGGCCAAAA	47(33)	25(12)	Silent
.02	3708	AGGTCGGGGT[C/T]GATGTCAACC	63(35)	9(8)	Silent
.03	3795	GGACCCACCT[C/A]CTGAAGATCC	62(35)	10(9)	Silent
524.01	1598	CACAAGTTGA[G/A]GAGGGCGATA	68(36)	4(4)	Silent
.02	2548	CTTATATTTC[T]^{10}GATGTCAACC	71(36)	1(1)	3'UT
.03	3158	AAAATTGTCT[GTTT]GTTTTCTCAT	50(34)	22(20)	3'UT
525.01	255	CTGCGGTTCT[C/T]GAGGGCGATA	54(34)	18(16)	Silent
.02	346	CGTGCCGGCT[C/T]TTCACCATCC	71(36)	1(1)	Leu->Phe
.03	523	CCCCATCCTC[A/G]TCCCGTGCCA	63(36)	9(9)	Ile->Val
1025.01	1051	CAACTAACCA[G/A]ACAACTGGGA	24(20)	48(44)	3'UT

.11	418	GCCCCTTTTG[C/T]AGCCCACGGC	6 (5)	5 (3)	N/D
.12	640	CAACTAACCA[G/A]ACAACTGGGA	15 (7)	7 (6)	N/D
1026.2	47	GTCTGGACGC[G/A]ACGGCGGCGG	2 (2)	3 (2)	5' UT
.9	262	CCCACCCCTT[G/A]GAGCACAAGA	28 (13)	4 (1)	Silent
.19	602	ATAAAGTATAGCGG[A/G]AGAGAN	5 (5)	11 (8)	3' UT
1027.2	405	TGGAAGAGAT[T/C]ATTGATGACA	2 (2)	2 (2)	Silent
.6	942	GGACAAAAAG[A/G]TATGACTCCA	8 (8)	4 (4)	Silent
.16	1361	CAGGAAGGCA[C/A]CCCTGAGGGG	13 (11)	3 (3)	Thr -> Asn
1031.31	2990	CCTTCGCCCA[G/A]CTGCGCCTCG	9 (7)	2 (2)	Silent
.32	2991	CTTCGCCCAG[C/G]TGCGCCTCGG	4 (4)	4 (4)	Leu -> Val
1032.1	3	AGTCGCCG[G/A]GGAGGACGGTCT	5 (4)	3 (3)	5' UT
.2	4	GTCGCCG[G/A]GAGGACGGTCTGC	5 (5)	3 (2)	5' UT
.3	69	CCGCCGCGGC[G/A]AAGATGGCCT	5 (5)	2 (2)	5' UT
.10	312	AAAAAGATTG[T/C]CGCTATGCTT	8 (8)	3 (3)	Silent
1037.20	2919	TGGTTATGGG[G/C]GTGCCAGAGG	15 (13)	2 (2)	3' UT
1038.5	723	CAGGTCCTGG[G/C]CCCCAAGCCT	7 (7)	3 (3)	Silent
.10	862	ACTCCAGCCC[C/A]TTTGCCCTTG	5 (5)	13 (10)	Silent
.13	1053	CCTCAGGGCC[G/A]TGAGAGTCCC	13 (10)	8 (7)	Arg -> His
1039.19	1665	ACCATGTCTC[A/G]GTTTATTTTT	2 (2)	6 (5)	3' UT
.23	1748	TATTTGAGTA[G/A]AAAATCACTT	3 (3)	2 (2)	3' UT
1040.7	2056	GCTGAAGAAG[T/C]CTTCGAGGCT	20 (16)	2 (2)	3' UT
1043.1	351	ACTTGAAGGA[T/C]GAAAGTGGCT	2 (2)	3 (3)	Silent
.2	372	TCAAAGATCC[C/T]TCCAGCGACT	2 (2)	3 (3)	Silent
1048.3	341	GCTACGCGAA[G/A]CTCTTTGCTG	2 (2)	2 (2)	Silent
1049.10	2648	CCTGAAACCC[T/A]GAAGCTGATG	5 (4)	3 (1)	3' UT
.12	2768	CAGTGGTAGC[G/A]ATGGAAAAAA	8 (6)	2 (1)	3' UT
1050.11	2381	CAGGAAGAAG[A/G]TATTCCAGGA	4 (2)	2 (2)	Ile -> Val
.13	2750	TTTTGCCAGC[G/A]TAGTGCTCCT	2 (2)	2 (1)	Val -> Ile
.14	3034	GAGTCCAGAG[T/C]GCTGCCAGGA	2 (2)	2 (1)	3' UT
1051.10	260	AGCTGGCAAG[C/T]TACTTTTCAG	15 (10)	3 (1)	3' UT
.18	409	TTTGCTTCTT[G/A]AGTAGAGCCA	17 (12)	3 (1)	3' UT
1052.7	428	TGTACAAATC[T/C]TTCATCCATA	7 (6)	2 (2)	3' UT
1053.24	4113	AGGAGAAGAC[C/T]TACCGGCGGC	8 (7)	8 (8)	Silent
1055.17	3122	CAGCGTCAGC[C/A]AGCTCAGCCT	4 (4)	4 (4)	3' UT
.23	3450	TGAGAAGGGC[T/C]TGGGACAAGA	26 (12)	3 (3)	3' UT
.25	3568	TCAAAAAACC[T/C]TTTTTTTCTG	26 (12)	2 (2)	3' UT
.01	2061	AGGCTGGTCG[C/T]GAACTCCTGA	61(34)	11(9)	3'UT
.02	2419	TTAAAAGATA[C/A]GCATGTCTTC	59(33)	13(10)	3'UT
.03	3047	TAAGTCTTTT[G/T]AGTGTCATCA	71(36)	1(1)	3'UT
.04	2960	TATTACTCAC[G/A]TATACCCCAT	71(36)	1(1)	3'UT
.05	3450	TGAGAAGGGC[T/C]TGGGACAAGA	60(33)	12(9)	3'UT
.06	3296	CTGCAAAGAG[T/C]GTACTGTGCT	60(33)	12(9)	3'UT
1056.12	407	CAAGAGCACC[G/C]GTGGGGCCCC	13 (9)	2 (2)	Val -> Arg
1057.20	3067	TAACTTTTCG[G/A]TCTTTCCCAT	7 (5)	3 (3)	3' UT
1059.11	1130	AACGTGAGTG[A/G]CATTTTCCGA	5 (5)	2 (2)	Asp -> Ala

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.19	1327	AATCATCCGA[G/A]GTCCTGAGGA	19 (14)	3 (3)	Val -> Ser
.27	1474	GGGAGGCCTG[G/A]GGCTGGGCCC	15 (11)	2 (2)	Gly -> Arg
1063.21	705	CGGACATGGC[C/T]CAGCTGGAGG	8 (7)	8 (7)	Silent
.22	721	GGAGGCCTGT[G/T]TGCGCTCTAT	16 (14)	2 (2)	Val -> Leu
.38	949	GCGTGCGTGA[G/A]GGCCCTGCCA	2 (2)	2 (2)	3' UT
1068.30	2756	GCGCCGCGGT[G/C]GCTACCGCCA	21 (15)	2 (2)	Ala -> Arg
1069.10	1199	GGGCGCCAGC[C/G]GAGTGCTTAT	17 (13)	2 (2)	Arg -> Glu
1070.3	303	AAGAGGATGG[G/T]CAGGAGTATG	3 (2)	6 (6)	Gly -> Val
.7	615	ACATTGGAGA[T/C]GATGATGAAG	6 (6)	2 (1)	Silent
.12	1092	GAAGTCTGCA[G/T]TTGAAGAAAA	5 (5)	3 (3)	3' UT
1072.20	1309	TCACGAGATT[T/C]GCCAGGGGCA	15 (10)	2 (2)	3' UT
.21	1310	CACGAGATTT[G/T]CCAGGGGCAT	4 (3)	5 (5)	3' UT
1073.2	65	GGCCCAGAGG[G/A]AATGGACCCC	2 (1)	2 (2)	Silent
1074.18	1428	TTGTGTGATT[T/C]CCTAAACATA	5 (4)	2 (2)	3' UT
.21	1650	TTGTCTTTTA[G/A]ACAACTAGAT	6 (6)	3 (3)	3' UT
.22	1652	GTCTTTTAGA[C/A]AACTAGATTT	5 (5)	3 (3)	3' UT
1077.19	1275	TATAATAATT[G/T]TATGGTACCT	3 (2)	3 (3)	3' UT
.22	1585	ATGTACATAA[T/A]TTTGAGGTAG	7 (5)	3 (1)	3' UT
.30	2336	TCAGGCACCC[A/G]TAGAAAGACC	4 (3)	10 (9)	3' UT
.34	2460	GAATTGGCCC[G/A]CTGGTACCAA	5 (4)	16 (14)	3' UT
1079.11	2035	CTGCTGTAGT[T/C]GCTCCATTCA	19 (14)	2 (1)	Silent
.18	2347	GCAACATCAC[A/G]TGGGCTGATG	25 (17)	2 (2)	Silent
1080.24	2367	TGCCTGAGGA[A/C]GGGCAGGGCC	1 (1)	5 (4)	3' UT
1081.17	805	GATTGATAGA[G/A]AGAAACTGCG	13 (8)	2 (1)	Ser -> Lys
.36	1178	ATGCATATTGTAAAATAAA[A/G]A	2 (2)	10 (9)	3' UT
1082.19	767	TTCGGGGCCT[C/T]CGCCGGCACC	7 (5)	2 (2)	Ser -> Phe
.27	924	ACGTGGACGA[C/A]CCCACGGGGA	3 (3)	3 (3)	Asp -> Glu
.40	1333	GTCTACAGAT[G/T]GGCTGTGGCC	4 (4)	5 (5)	3' UT
1088.11	112	CCGAGGGGGA[C/T]GCGCTGGATG	23 (16)	7 (5)	Silent
.12	144	AAGCGCTACT[G/C]CTGCCGCCGG	24 (18)	5 (4)	Cys -> Ser
.13	145	AGCGCTACTG[C/G]TGCCGCCGGA	21 (16)	5 (4)	Cys -> Trp
.20	226	GACCACGCTG[A/G]AACCCACCCA	23 (16)	18 (11)	3' UT
.21	238	ACCCACCCAC[C/A]CGCTGTGCTG	31 (19)	3 (3)	3' UT
.24	270	TGAGCGTCCT[A/G]CCCCGAATTC	29 (18)	9 (6)	3' UT
.27	338	GTGTGTATCC[C/G]ATACCCCACT	23 (15)	2 (2)	3' UT
1090.18	4153	GTGTAAAATA[T/C]GCTGCTTGGA	13 (12)	2 (2)	3' UT
.21	4215	CTCACAGTAA[T/C]CTTCACACTT	21 (16)	2 (1)	3' UT
1091.3	793	AGGATCCCCC[A/G]CCGCCTATGG	2 (1)	5 (2)	Silent
.9	962	CTTTCTTGTG[C/T]CCCTTCTGAG	4 (3)	5 (2)	Pro -> Ser
.14	2078	AAGAGGTGCA[A/G]TGTGATCTGA	6 (5)	11 (8)	3' UT
1092.5	342	CCTGGAGGCG[G/C]CCAACGGCGA	16 (8)	4 (1)	Ala -> Pro
.10	401	GGCCTGGGCC[C/T]TCCCGCGACT	9 (6)	11 (5)	Silent
.11	503	AGATCGACAA[C/T]GCCCGTCTGG	11 (6)	6 (5)	Silent
.22	1034	TTGGAGCCCA[G/C]CTGGCGCATA	4 (4)	3 (2)	Gln -> His

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.23	1035	TGGAGCCCAG[C/G]TGGCGCATAT	3 (3)	3 (2)	Leu -> Val
1093.2	258	CTCTCACAGA[C/T]GAGATCAACT	3 (2)	2 (1)	Silent
.3	330	CAGACACATC[T/C]GTGGTGCTGT	3 (2)	3 (2)	Silent
.4	339	CTGTGGTGCT[G/A]TCCATGGACA	3 (2)	3 (2)	Silent
.6	420	TTGCTCAGAG[A/G]AGCCGGGCTG	3 (2)	3 (2)	Silent
.22	954	GCGTTGGAGG[T/C]GGCTTCAGTT	7 (2)	3 (1)	Val -> Ala
.23	960	GAGGTGGCTT[C/T]AGTTCCAGCA	7 (2)	3 (1)	Silent
.24	972	GTTCCAGCAG[T/C]GGCAGAGCCA	7 (2)	3 (1)	Silent
.27	983	GGCAGAGCCA[T/C]TGGGGGTGGC	7 (2)	3 (1)	Ile -> Thr
.28	1065	GGAAGAGCTA[T/C]AAGCACTAAA	9 (3)	3 (1)	Silent
.44	1198	TAGAGCTGGG[G/T]ATGAATGCTT	13 (2)	3 (1)	3' UT
.45	1202	GCTGGGGATG[A/G]ATGCTTAGTG	13 (2)	4 (1)	3' UT
.49	1579	TGTGCTCTTC[A/G]CTCTTTGCAA	14 (3)	5 (2)	3' UT
.50	1582	GCTCTTCACT[C/G]TTTGCAATTG	13 (3)	6 (3)	3' UT
1094.24	3103	TGCTTTTGCT[C/G]GCTTTGGCCA	15 (9)	4 (2)	3' UT
.25	3104	GCTTTTGCTC[G/C]CTTTGGCCAG	2 (2)	4 (2)	3' UT
1095.17	2885	CGTAGGAAGG[G/C]CCTCAGTGAA	18 (11)	2 (2)	Silent
.25	2994	GTGGACTCCT[G/T]GGAGCTCCTG	14 (10)	3 (3)	3' UT
.31	3246	GGGGATGAAA[C/A]CCCAAGGGGC	10 (7)	12 (11)	3' UT
1098.10	1486	GGCAGTGGCC[G/C]CCCTGGGTGA	8 (7)	3 (3)	Ala -> Pro
.13	1522	CACGTATGAG[G/C]ACATCCAGAC	2 (1)	12 (10)	Asp -> His
.21	1740	TGCATTCTTT[T/C]GGAACTCAAT	11 (6)	2 (2)	3' UT
.25	1850	GGAGGGCGGT[C/T]GGTGCTTCCC	21 (13)	2 (2)	3' UT
.29	1942	TGACCTATCA[A/G]AGCCTCCCGG	16 (11)	6 (5)	3' UT
.35	2029	CCAAGGAGCG[C/A]GCTCCACGCG	13 (10)	2 (2)	3' UT
1099.36	7590	TGGTTTGAGA[G/C]CTGGCGCTAC	12 (11)	6 (4)	3' UT
.37	7591	GGTTTGAGAG[C/G]TGGCGCTACC	9 (8)	6 (4)	3' UT
.44	7705	ATGGATCTGA[C/T]CCCTGTCAGA	13 (12)	9 (8)	3' UT
.01	215	ATTCCTCAGT[C/T]CTTCATGATG	63(36)	9(9)	Ile->Val
.02		Nucleotide repeat	66(35)	6(5)	3'UT
1100.16	3865	ATTGGGTCCT[C/G]AGCCTTCTGG	4 (3)	4 (3)	3' UT
.17	3904	GGACAAAGCC[T/C]TTTCATCTGA	2 (2)	4 (3)	3' UT
.19	3994	GGTGGAGTTC[T/C]TCCATGCAGG	6 (6)	6 (5)	3' UT
.22	4046	TATCCGAGGT[G/T]CTGCCGGGGC	6 (6)	5 (5)	3' UT
1102.29	1967	TAACTTGGGT[T/G]TGAAAAAAAT	2 (1)	25 (20)	3' UT
.30	1982	AAAAATAAAA[T/G]TCCTAAATTT	2 (1)	24 (20)	3' UT
.31	1991	AAAAATAAAATTCCTAAAT[T/C]T	2 (1)	21 (17)	3' UT
1105.15	2038	GGGCCTGCCT[G/C]TGAGTGGTGC	3 (3)	6 (6)	3' UT
1109.4	884	AGCTTGCCTG[C/T]TTCAGCAAAA	4 (4)	2 (1)	3' UT
1110.11	6466	CTGATGCAGA[T/C]TCTTGTCTTG	5 (5)	5 (5)	3' UT
1111.8	794	AAGACGGCTA[T/C]GAGTTCTTTG	2 (1)	7 (6)	Silent
.15	1087	CTGCCATGCT[G/T]GGGGGGGGTC	8 (5)	4 (4)	3' UT
.16	1110	CCCGACCCCT[A/C]AGGCCCACCT	3 (1)	18 (17)	3' UT
.17	1146	GAGCCTTGGT[G/T]TATTTTTCTT	22 (18)	4 (4)	3' UT
1114.18	540	ATGCTACCTA[C/T]CGGGAAGGCA	29 (16)	2 (2)	Silent
.20	585	TCACTGCCAA[T/A]GCTCTCGCTT	22 (15)	6 (4)	Asn -> Lys
.21	586	CACTGCCAAT[G/T]CTCTCGCTTT	16 (12)	6 (4)	Ala -> Ser
.27	704	CCCAAATTCG[C/T]CGTTGCCACT	20 (14)	3 (3)	Ala -> Val
.01	177	GAACAACCAC[T/C]GGGTCCTACA	70(36)	2(2)	Silent
.02	328	ACTGAATGAG[C/G]CTCCACTGGT	71(36)	1(1)	Pro->Ala

.03	328	GGCCGGAGGC[A/G]TTCACTCCAG	30(20)	42(32)	Silent
1115.2	77	ACTGCCGCAG[G/A]AATGCCGTCT	13 (9)	4 (1)	Silent

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.5	130	CTTCCAAAGG[T/C]CCGGAAAACT	8 (7)	14 (4)	Val -> Ala
.15	643	TTCAACGACC[T/C]GGGCTCCGGA	11 (8)	2 (1)	Leu -> Pro
.16	732	CAAGAAGGGG[A/C]CCAGGCTTGG	12 (7)	4 (2)	Thr -> Pro
1116.2	121	CGGACCGTCC[T/A]GACTACAGTT	2 (1)	4 (4)	Silent
.3	173	CCGGGGAATG[A/C]AGCCCACAGA	2 (1)	5 (5)	Lys -> Gln
1117.1	15	CCTGCAGCCC[T/C]GGCCTTCCGC	10 (7)	4 (3)	5' UT
.2	16	CTGCAGCCCT[G/T]GCCTTCCGCC	10 (7)	4 (3)	5' UT
.5	19	CAGCCCTGGC[C/T]TTCCGCCACC	10 (7)	2 (2)	5' UT
.19	401	TGGCAGCCTT[G/T]GCCAAGGCCC	12 (7)	8 (4)	Leu -> Phe
.01	1287	GCCATGCACT[C/G]ACCAACGCCA	65(36)	7(7)	Ser->Val
.02	3385	TTGCCTGGAC[G/A]TTGGCCTGCG	70(36)	2(2)	3'UT
1118.5	1681	GACATGGTTG[G/A]TTATGCACAA	6 (5)	2 (1)	Val -> Asp
.28	2945	ATGATTAAGG[A/G]CCAGAGGATC	7 (6)	7 (5)	3' UT
1119.11	1075	TCACAAATTA[G/A]GCCACGGCCC	3 (3)	3 (3)	3' UT
1121.17	1524	CATCCGTTGC[A/G]TATGGCTGCA	3 (3)	2 (2)	Silent
.23	1669	TGCACGTCTG[G/C]CCAATATTGA	6 (6)	3 (3)	Ala -> Pro
.27	1902	GACAGACTGG[G/A]AAAATATTGA	2 (2)	20 (17)	Gly -> Glu
1123.9	2485	CCTGATATGA[A/C]TGTTACTAAA	5 (5)	4 (4)	Asn -> Thr
.17	2807	TTGACATAAC[T/C]ATCTTTTTGA	4 (3)	3 (3)	3' UT
1124.2	119	TCTTATCGGA[G/A]CTTGTATGTG	2 (1)	3 (3)	5' UT
.7	3616	TACTCCATAC[G/T]CACTTCAAGC	2 (1)	5 (3)	Ala -> Ser
1127.2	4	TGCAAAA[G/A]CGCAGGATCAAGG	13 (8)	2 (1)	Ala -> Thr
.15	75	TCAACATCTG[T/C]GTTGGGGAGA	22 (14)	2 (1)	Silent
.34	339	AGGAACACAT[T/C]GATCTGGGTA	2 (2)	31 (16)	Silent
1128.9	483	AAATAAAAAAAAA[A/C]AAAACCC	4 (3)	4 (3)	3' UT
.10	484	AAATAAAAAAAAAA[A/T]AAACCC	4 (3)	4 (3)	3' UT
1130.7	248	CCCCCTGCGG[G/T]TGAAGAACTT	25 (12)	9 (4)	Val -> Leu
.11	320	GGAATACCGG[G/T]ACCTGACCAC	26 (12)	2 (1)	Asp -> Tyr
.13	364	ACCGAGACAT[G/T]GGTGCCCGGC	15 (10)	3 (2)	Met -> Ile
.16	377	TGCCCGGCAC[C/G]GCGCCCGAGC	16 (8)	4 (3)	Arg -> Ala
.19	421	TGGAGGAGAT[C/T]GCGGTCAGCA	12 (7)	2 (1)	Silent
1131.12	502	TGGCTGACCA[G/A]GCTGAGGCCC	18 (13)	2 (2)	Silent
1133.20	279	CTGAGTCTGC[C/T]ATGAAGAAGA	41 (18)	2 (1)	Silent
.35	517	CCTAATTCTG[A/G]ATATATATAT	19 (12)	4 (2)	3' UT
1135.22	301	AAAACAAGAC[T/G]GGGGCTGCTC	38 (20)	8 (4)	Silent
.23	343	CGGGCTACTA[C/T]AAAGTTCTGG	40 (18)	4 (2)	Silent
.32	438	AAGAGTGTTG[G/A]GGGGGCCTGT	32 (18)	2 (2)	Gly -> Ser
1136.1	13	CGCCGCTGCG[G/A]AGGGAGCCGC	9 (9)	10 (6)	5' UT
.16	190	GGAGCCGGCA[G/A]CCGACGGCAA	31 (21)	5 (4)	Ala -> Thr
.18	197	GCAGCCGACG[G/C]CAAAGGTGTC	32 (23)	5 (5)	Silent
.19	198	CAGCCGACGG[C/A]AAAGGTGTCG	21 (16)	8 (5)	Ala -> Glu
.23	243	GCCAGCGGAA[G/C]CCTGCCACCT	31 (20)	5 (5)	Lys -> Asn
.24	244	CCAGCGGAAG[C/G]CTGCCACCTC	31 (20)	5 (5)	Pro -> Ala
.25	245	CAGCGGAAGC[C/T]TGCCACCTCC	31 (22)	6 (3)	Pro -> Leu
.29	283	CAACAAGAAT[G/C]CTCGCGCCAC	26 (18)	5 (5)	Ala -> Pro
.30	284	AACAAGAATG[C/G]TCGCGCCACG	26 (18)	5 (5)	Ala -> Val
.32	286	CAAGAATGCT[C/T]GCGCCACGCT	31 (22)	2 (2)	Arg -> Cys
.41	387	TCCTGCGCAC[G/C]CAGAAGCCTG	2 (2)	19 (14)	Silent

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1137.1	3	CTTCCTTC[G/T]AGGAGGTGGCAG	2 (2)	3 (2)	5' UT
.15	331	GTGCCGAGAT[C/T]GCTCACAATG	22 (12)	4 (2)	Silent
.23	419	CAATGCCAGG[C/G]TGCGCAGTGA	13 (9)	3 (2)	Leu -> Val
.25	488	TAAAAACTGC[C/A]ATCTGGCATC	8 (8)	4 (4)	3' UT
1138.8	78	AGGAGGAGCT[G/T]CTGAAACAGC	30 (17)	2 (2)	Silent
.14	127	GCTGCGCGTC[G/A]CCAAAGTGAC	31 (15)	2 (2)	Ala -> Thr
.24	354	AGCAGCAGCG[G/T]AAGGAGCGGC	28 (16)	2 (2)	Silent
1139.21	334	TTCCGAAGCA[A/G]TCTTCCTGCT	33 (20)	3 (1)	Asn -> Ser
1140.3	17	CCGCTGCTCG[C/A]CATGTCTTCT	22 (15)	3 (2)	5' UT
.20	341	AATATGTAAG[G/A]CCTTTCTTTT	32 (16)	2 (2)	3' UT
1141.5	201	ATCAGACTAG[A/T]GCTGAGTCTT	2 (1)	11 (5)	Arg -> Ser
.7	346	GCGCCGTTGG[C/A]ATCGTAGAGT	4 (3)	3 (2)	His -> Asn
.18	1071	GGATAAGGCA[G/A]CTGCTGCAGC	5 (4)	6 (3)	Silent
.21	1376	TGTTATACAGGCAGTGA[G/A]AAA	14 (10)	5 (4)	3' UT
1142.13	556	CTTGTGACTG[A/G]CCTCTGGTCC	8 (7)	3 (3)	Asp -> Ala
1143.17	470	ATCTACAAGC[G/T]TGGTTATGGC	32 (20)	2 (2)	Arg -> Leu
1144.1	211	GCCGCGGCGC[G/C]CCCCTCGCCA	7 (5)	4 (4)	Silent
.5	286	CCGCCGAGGG[C/A]ATTCACACGG	11 (9)	5 (4)	Ala -> Glu
.6	287	CGCCGAGGGC[A/T]TTCACACGGG	15 (13)	4 (3)	Ile -> Phe
.17	494	TGTGAAGCTG[C/T]CCTCCGGCTC	9 (8)	2 (2)	Pro -> Ser
.26	700	ACCAGCACAT[C/T]GGCAAGCCCT	24 (18)	2 (2)	Silent
1145.18	395	GTGAAAAATA[C/T]ATCCGCAGGG	21 (14)	7 (7)	Silent
.20	405	CATCCGCAGG[G/T]TTCGGATGAG	27 (20)	2 (2)	Val -> Phe
1146.16	276	TGTTTGCAAA[G/T]GCCCTGGCCA	16 (12)	3 (3)	Lys -> Asn
.18	285	AGGCCCTGGC[C/A]AACGTCAACA	13 (10)	5 (5)	Silent
.22	340	ACCTGCTCCA[G/C]CAGCTGGTGC	16 (12)	3 (3)	Ala -> Pro
.23	341	CCTGCTCCAG[C/G]AGCTGGTGCT	15 (12)	3 (3)	Ala -> Glu
.25	343	TGCTCCAGCA[G/A]CTGGTGCTGC	17 (12)	2 (2)	Ala -> Thr
1147.22	324	GAGACTGGCA[G/A]GCCTCGGCCT	7 (5)	3 (3)	Arg -> Lys
1148.29	390	TCGGTGACAT[C/T]GTCACAGTGG	33 (17)	3 (2)	Silent
1149.14	174	GAACCGGGGC[C/G]TGCGGCGGAA	14 (12)	3 (2)	Leu -> Val
.22	414	CGTAAAGCAT[G/T]GCCGGCCCGG	23 (20)	4 (3)	Ala -> Cys
1150.20	257	CTCAAAGACC[T/C]GGAAAAATGG	42 (19)	2 (1)	Leu -> Pro
.34	435	CCTCATGGAC[T/A]AAAAAAAAAA	7 (6)	4 (3)	3' UT
1151.13	312	TCCAAAGCCC[T/C]GGTGGCCTAT	33 (16)	6 (1)	Leu -> Pro
.14	313	CCAAAGCCCT[G/T]GTGGCCTATT	33 (16)	6 (1)	Silent
.16	346	TGGATGAGGC[T/C]TCCAAGAAGG	34 (16)	2 (1)	Silent
.22	439	AGTTTGGAGG[C/T]CCTGGTGCCC	20 (14)	6 (4)	Ala -> Val
.25	517	TAATAAACAG[T/A]TTTTGAGGGA	23 (15)	3 (1)	3' UT
1152.15	131	GCGCGTGTGC[G/A]AGGAGATCGC	34 (18)	3 (2)	Ser -> Lys
.19	160	CCAGCAAAAA[G/C]CTCCGCAACA	31 (18)	6 (4)	Lys -> Asn
.20	161	CAGCAAAAAG[C/G]TCCGCAACAA	29 (16)	5 (3)	Leu -> Val
.24	184	TAGCAGGTTA[C/T]GTCACGCATC	20 (9)	22 (15)	Silent
.31	379	CCAACCTTCA[G/A]GTCACTCAGC	36 (23)	2 (2)	Silent
1154.8	119	GGGCACAGCC[C/T]TAAAGGCCAA	17 (9)	3 (2)	Silent

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.39	477	TAGTAATAAA[T/C]TTTCATATGC	21 (15)	2 (2)	3' UT
1155.6	64	TATTCTCCGA[G/C]CTTCGCAATG	29 (19)	3 (3)	5' UT
.7	65	ATTCTCCGAG[C/G]TTCGCAATGC	25 (17)	3 (3)	5' UT
1157.3	75	TGGGCAGGAC[C/G]GGTTCTCAGG	18 (11)	3 (3)	Silent
.12	290	GTCTGTCACA[A/G]TCTGCTCCTT	28 (12)	11 (7)	3' UT
1158.4	55	CGAAAATTCG[G/A]CCAGGGTTCT	36 (20)	2 (1)	Ala -> Asp
1159.2	68	AGCACCAGCG[G/T]TGGCAGAGAC	24 (14)	2 (1)	Val -> Leu
.7	199	ACAGTGCAGG[G/A]CGGTATGCCG	16 (10)	5 (3)	Gly -> Glu
1160.10	124	TCAGGGAGCT[G/A]AATATTACGG	28 (18)	2 (1)	Glu -> Lys
.15	166	GTGGTGGTCG[G/A]AAAGCTATCA	28 (17)	2 (2)	Glu -> Lys
.17	229	TCCAAGTCCG[C/G]CTAGTACGCG	2 (2)	29 (19)	Pro -> Ala
1161.8	263	AAGGCAACGC[C/T]CTGCTGCGGC	30 (16)	2 (2)	Silent
.9	264	AGGCAACGCC[C/T]TGCTGCGGCG	22 (14)	9 (9)	Silent
.11	283	CGGCTGGTCC[G/C]ATTGGGGGTG	13 (9)	4 (4)	Arg -> Pro
1163.8	1522	GTACTTCCTC[G/T]TCCTCATGCC	2 (2)	5 (1)	Arg -> Leu
1165.1	97	CCACGACCGT[G/C]GCTATCTGGT	3 (3)	2 (2)	Ala -> Arg
.4	180	GTGAGGGGCG[G/T]CCGCGGCGCA	4 (3)	4 (2)	Silent
.7	273	CCAAGGTGGG[C/A]ATCAAGACCA	10 (7)	4 (3)	Ala -> Glu
.8	274	CAAGGTGGGC[A/T]TCAAGACCAT	20 (12)	3 (2)	Ile -> Phe
.13	429	AGCAGGAGCT[G/C]CTCATCAACA	8 (7)	5 (4)	Silent
.14	430	GCAGGAGCTG[C/T]TCATCAACAT	5 (5)	8 (5)	Leu -> Phe
.29	901	CCCCCAGAGG[G/A]AGGTCACCTG	13 (10)	4 (3)	3' UT
.35	1007	GCTTCCTCCT[G/T]GGCCCTCAAT	6 (5)	4 (4)	3' UT
.38	1189	GATGTTTTGA[C/G]GAAATAAATT	2 (2)	7 (6)	3' UT
1170.2	410	ATTGCGAATC[G/C]TTAGATATCC	2 (2)	2 (2)	Val -> Leu
1171.27	2823	AAGAGATGAA[A/T]AAAAAAAAAA	8 (6)	4 (4)	3' UT
1172.15	1519	CTCTAGTGTT[G/C]AGGGATGTAG	7 (7)	2 (1)	3' UT
.19	1784	CAGGTCTTAA[T/C]GCCTCCATAC	3 (3)	2 (2)	3' UT
.25	2423	GAGAGACTGG[T/A]GGGTCTGTCT	7 (6)	5 (4)	3' UT
1173.12	4730	AGTAGGTAGG[G/T]CTAGTAGGTA	6 (6)	2 (1)	3' UT
.01	981	GCAGCCCCAG[T/C]GCACCTGAGC	24(18)	48(30)	Silent
.02	1041	ACATCAAGAG[A/G]TACCTGGGCG	71(36)	1(1)	Silent
.03	2400	AGCTGAGTGC[C/T]GCCACCACCT	71(36)	1(1)	Silent
.04		4 bp deletion			
.05	2567	CTAGATAGCA[A/G]ATAGCTCTCA	71(36)	1(1)	3'UT
.06	2888	CCCAAGCTGC[C/T]TCATGGCCCG	63(36)	9(9)	3'UT
1174.24	3200	TGTTGACAGG[G/C]TTTTTAAGAA	10 (8)	2 (2)	3' UT
.27	3302	TCTGCCCAAGC[A/C]AAAAAAAAA	5 (3)	3 (2)	3' UT
1176.13	2571	GAGGCTTTGC[C/T]TTGCCTGCAT	6 (4)	3 (3)	3' UT
1177.18	1684	CTCTTCCCCC[T/C]AAAAATGGTA	13 (10)	3 (3)	3' UT
.21	1864	GTTAGCTTTA[A/G]AAAAAAAAAA	5 (5)	3 (3)	3' UT
1181.8	678	TACCAAAGCA[G/A]GGGTTCCCCA	10 (7)	2 (2)	Arg -> Lys
1183.18	1719	CTTCCTGCTC[G/A]ACTGAAAAAA	14 (9)	2 (1)	3' UT
.21	1799	TGGCTTTCAG[G/C]CCTGGCCTTT	15 (10)	5 (4)	3' UT

1184.14	2292	GCCTAAATGT[G/T]TGAAGTGCGA	30 (18)	2 (2)	3' UT
1186.7	1337	GGGAGAGGTG[A/G]CCCTGAGGGA	2 (1)	4 (3)	3' UT
1188.7	1601	AGTCATCTGA[G/A]GTTATGCTTT	4 (3)	2 (1)	3' UT

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1189.13	1270	CGGAAAGGAA[G/A]CGTTGGCAGC	11 (9)	3 (2)	3' UT
.16	1341	AGCCCCAGGG[A/G]CCAATTTTCC	14 (12)	2 (1)	3' UT
1190.5	1010	GGGGTTGGGC[G/T]GGTTCCTTTG	2 (2)	3 (3)	3' UT
1193.1	79	CTCTCCCCTC[C/G]AATCCTATCC	5 (5)	2 (2)	5' UT
1196.23	2123	TATGTTTTCC[T/C]ATGCAATAGT	19 (14)	2 (2)	3' UT
1198.29	2395	TGGCAAAGTC[T/C]GAAATAGGTC	20 (15)	4 (2)	3' UT
1199.3	1012	AGATTCAGAA[C/T]ATGGTGGGGA	3 (2)	2 (2)	Silent
.13	1460	TGAGAACACC[G/C]CGCAGCGTGA	8 (7)	2 (2)	3' UT
1202.7	671	ACCATAACTT[T/C]TTTTTAAGGA	13 (7)	11 (6)	3' UT
1205.1	942	GGAGAAAATT[G/A]AAGAATATCT	13 (6)	2 (1)	Glu -> Lys
1206.3	740	ACATCACAAA[A/G]CAACCTGTGG	3 (3)	2 (1)	Silent
1208.3	1984	TATTCCGTAC[G/A]TACAATGCCT	2 (1)	2 (2)	Silent
.15	3163	AATTTTTTTT[T/C]TTTTTAATTA	2 (1)	15 (6)	3' UT
1214.9	1566	GCATCCTGGA[C/T]AGCAACAAGA	5 (3)	2 (2)	Silent
1216.8	202	AGCGGAGCGC[C/G]TCCCGGGACA	5 (4)	3 (2)	Silent
1217.3	2545	GCCTCTCGGC[C/T]TTTCTCCACG	5 (3)	2 (1)	Silent
.5	2688	GCCGTGTGCC[C/A]ATGCTACCCT	12 (6)	3 (3)	3' UT
1218.10	2757	GCAGGCTGCC[C/T]TTTAGAGAGG	4 (2)	2 (1)	Silent
.01	1100	GATGTCAGTG[G/C]CCCCATGCCC	71(36)	1(1)	Gly->Ser
.02	1287	GCCATGCACT[C/G]ACCAACGCCA	71(36)	1(1)	Silent
.03	3385	TTGCCTGGAC[G/A]TTGGCCTGCG	71(36)	1(1)	Silent
1221.20	1893	TGGAGCCTTC[G/T]GCTGGAAGTC	9 (7)	3 (2)	3' UT
1222.30	2797	CACAAACCCA[A/G]TTGTAAATAA	14 (11)	2 (1)	3' UT
1223.3	2813	AAGCAGGAGG[C/T]TAAGAAAGTG	13 (10)	2 (1)	N/D
.9	3662	GGACCGCAGT[C/T]CAGCATTTGT	2 (2)	2 (1)	N/D
.10	3727	TAAACTGAAG[T/A]GTGTTTTTCC	4 (4)	3 (2)	N/D
.15	3855	ACGTCCCAAC[G/A]AAGAGACCAC	24 (19)	2 (2)	N/D
.16	4110	CACCTTGGTG[G/A]AGAACAAGAA	20 (17)	2 (2)	N/D
.20	4155	CGACGTGGAT[C/T]CCATCGAGGT	21 (17)	2 (2)	N/D
1224.13	1739	GCAGAGCCAC[C/A]AGGGAAAAGT	2 (2)	2 (2)	3' UT
.17	1936	CCTCTTCTAA[T/C]CTCAAGGGTC	3 (2)	8 (7)	3' UT
.21	2061	GCGAGTGAGT[G/T]GAGAGCCAGC	15 (11)	17 (13)	3' UT
.22	2079	AGCTCTGCGG[A/G]GTCATCACGC	15 (11)	17 (13)	3' UT
1227.9	1107	AGAAGGTGAA[C/A]CCCCTGGGGG	9 (6)	4 (3)	Asn -> Lys
.16	1207	TGGGAAGAGG[G/C]CATACGGAGT	20 (14)	2 (2)	Ala -> Pro
1229.18	1919	ACTCCGTGCG[C/T]AATGCCGTCA	4 (3)	2 (1)	Silent
1235.11	1194	TAGCCGCCAG[G/A]ATTGCCATGA	18 (12)	2 (2)	Asp -> Asn
1238.14	1133	AGAACCTGAA[G/A]GCTGCGCAGG	6 (4)	2 (2)	Silent
.17	1298	AACAACTCCA[G/A]GCCCTGCCCC	8 (6)	2 (1)	3' UT
1239.13	1289	ACTTTTCCTC[T/C]AATCCTGGAA	11 (5)	7 (4)	3' UT

.14 1292 TTTCCTCTAA[T/C]CCTGGAAATT 16 (7) 2 (2) 3'-UT

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1241.13	1802	AATTAAAGTTTTTCTTC[C/T]ATG	10 (7)	2 (2)	3' UT
1242.18	3296	TCCTGTCACA[T/C]GTGCAGCAGG	13 (11)	2 (2)	3' UT
.20	3328	AGCGGGCATC[G/T]CTGCCGCCAT	7 (7)	3 (3)	3' UT
1243.5	134	GAACGCAGTG[G/A]ATGCCTTTCG	4 (4)	3 (3)	Asp -> Asn
.6	184	TGCGCAGCCC[C/G]GTCACCAACA	7 (7)	3 (2)	Silent
.7	185	GCGCAGCCCC[G/T]TCACCAACAT	7 (7)	4 (2)	Val -> Phe
.24	1528	CGGTGGAGCA[G/A]CCCCTGGGCT	10 (8)	3 (2)	3' UT
.31	1789	TACACGTGTT[G/A]CTTCGTCCAG	14 (9)	2 (2)	3' UT
.32	1790	ACACGTGTTG[C/A]TTCGTCCAGT	16 (9)	8 (7)	3' UT
1246.6	1512	ATCCCGGAGG[G/T]TCACTCTGAA	2 (2)	2 (1)	Val -> Phe
.9	1958	ACGTTTTAAC[A/G]TAGTAAATCC	3 (3)	6 (6)	3' UT
1247.6	517	GCGGACAGTA[C/T]ATTGCCATTG	2 (2)	2 (2)	Silent
1248.4	164	TGATGTCCCC[C/T]TTCGACCCGT	4 (3)	2 (2)	Silent
.5	172	CCCTTCGACC[C/A]GTCGGGTTTG	2 (1)	3 (3)	Pro -> Gln
.11	815	AGCACAGCCC[C/T]TCTACCAGGG	13 (7)	2 (2)	Silent
1249.1	50	ACCGCCTGCG[G/A]AGTAACTGCA	4 (3)	2 (2)	5' UT
.26	1800	TTGTAAAAGG[G/T]TTACTCTCAT	26 (16)	2 (1)	3' UT
1250.1	353	GCCCCGCCAG[G/A]ATTAACACAG	3 (2)	2 (2)	Silent
1251.11	1070	CCGCCAACGG[C/A]AACATCGACC	2 (1)	4 (2)	Ala -> Glu
.18	1974	CTGGGAAATG[C/A]GGGACTGGAA	2 (1)	2 (2)	3' UT
1253.7	673	GCCAGGTGGT[G/C]CAGATCCCTG	2 (2)	2 (1)	Silent
.11	1620	GCCTATGTCG[G/A]CGACGTCCAC	2 (2)	2 (1)	Ala -> Asp
.13	1672	ACACCAAGAC[C/T]ATGGAGCTGC	2 (2)	2 (1)	Silent
.16	3427	TCGACCACGC[G/A]GAGCGGGAGC	2 (2)	2 (1)	Silent
.21	3848	GACCCCGCTG[C/T]CACCCGCTTT	2 (2)	2 (1)	3' UT
1255.11	895	TCAAATGAAT[C/G]AACCACCTGG	2 (2)	2 (1)	Gln -> Glu
.23	1729	TCATTTTTCT[A/G]TATAGGCTGC	2 (2)	17 (8)	3' UT
.24	1731	ATTTTTCTAT[A/G]TAGGCTGCAC	2 (2)	17 (8)	3' UT
.27	1801	TTTCCAATAAAATC[G/A]GAATTC	3 (2)	3 (3)	3' UT
1257.11	674	AACAAGAACA[C/T]ATGATAAATT	9 (6)	2 (1)	Silent
.19	954	GTGAGAGAAC[G/C]AAATCTCTAT	21 (14)	3 (2)	3' UT
.20	955	TGAGAGAACG[A/C]AATCTCTATC	19 (14)	3 (2)	3' UT
1258.11	329	ATCACAGCAA[A/G]AGAGAGGTTC	22 (9)	4 (1)	Lys -> Arg
.15	357	TCACTACCAA[C/T]CTGATCAATT	24 (10)	6 (3)	Silent
.17	422	TCTGCCTTTT[C/T]TACCATGATG	25 (11)	2 (1)	Ser -> Phe
.20	533	AGCTTCCTAA[G/A]TCAAGGCCAA	27 (13)	2 (1)	Ser -> Asn
.32	745	GCTTCCAGAA[C/G]AGATCAAAAA	17 (10)	2 (1)	3' UT
1261.6	425	CTGGCATCAT[C/T]GCCATCTACG	9 (3)	2 (1)	Silent
.20	908	CGCCCCTCCA[G/A]GCCCCCGGCG	8 (3)	3 (3)	3' UT
1265.1	46	ACTCGAGCCT[G/A]CTGTTCACCG	3 (2)	2 (1)	5' UT
.19	1023	GGAGGGGGCA[A/G]ATGGTGGTTG	2 (1)	20 (7)	3' UT
1266.1	343	CGCTGCGGAC[G/A]AAAAGGCCAA	2 (2)	3 (2)	Glu -> Lys
.7	661	AGCAGGTGAA[G/A]GGCATCGCTG	7 (6)	4 (3)	3' UT
.9	671	GGGCATCGCT[G/T]CCCCAGGCCT	10 (9)	4 (3)	3' UT
.16	865	GTAGAGCACA[G/A]GGGTTTCCCC	25 (12)	2 (2)	3' UT

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1267.11	1776	GGCTAGAGGA[T/C]GCACGGTGGC	2 (2)	7 (5)	3' UT
1268.10	6529	TTCATCCTCA[C/T]TCCCCACATC	10 (6)	2 (2)	Thr -> Ile
1269.19	1893	CAACTTCAAC[C/G]TGGAGGTGCA	12 (4)	3 (3)	3' UT
.20	1941	TAAAAAGGTG[A/G]CTGTTTTATA	12 (4)	4 (4)	3' UT
1270.11	331	TTGTCCTCAG[T/C]ACCTCTCCGT	11 (9)	2 (2)	5' UT
1271.14	949	GGGTGTATTA[T/C]CCAGGTACTC	18 (11)	5 (1)	3' UT
1272.10	2678	TGTTAAGGAA[C/T]GCTAGCAGGG	3 (1)	3 (1)	3' UT
1273.13	3127	AAAGGAAGTT[T/C]TCCTTTTGAA	7 (2)	10 (3)	3' UT
1274.16	2696	ATATTTTTTC[A/G]TAATCTATAT	7 (6)	3 (2)	3' UT
1278.7	864	AGTGTGACCC[G/A]GACTGCCTCC	3 (1)	2 (2)	Silent
.32	3897	CCAGAACACG[G/C]CTCACGCTTA	5 (3)	3 (3)	3' UT
.33	3898	CAGAACACGG[C/G]TCACGCTTAC	4 (3)	4 (4)	3' UT
.34	4013	TGTTGTGTGT[A/G]TCGAGAGGCC	10 (7)	3 (2)	3' UT
1280.5	1648	TTAAGAGGAC[G/A]TAATGGGGTC	14 (8)	4 (3)	3' UT
.15	1957	TAAAGATGATTGTGG[G/A]AATTC	2 (2)	9 (8)	3' UT
1282.1	2155	TTTGGTGGGC[C/T]TACTTGGTGC	7 (3)	6 (1)	3' UT
.2	2283	GTGTGGCGTA[G/C]GCAGTGGGTC	13 (1)	2 (2)	3' UT
.9	2799	TTACATCACC[G/A]CCACTACTGC	6 (3)	2 (2)	3' UT
.10	2824	CAGTGCCCAG[T/C]GGCCGCATGC	4 (1)	3 (3)	3' UT
.15	2937	TGGTTTTGTT[G/C]CCTGACACAG	11 (4)	3 (1)	3' UT
1284.1	249	CTGTCGACGA[T/C]CCCTACGCCA	7 (7)	4 (3)	Silent
.6	522	GGGGCAGTGC[G/C]GTCATCTCCC	5 (1)	5 (4)	Silent
.7	523	GGGCAGTGCG[G/T]TCATCTCCCT	7 (4)	4 (1)	Val -> Phe
.10	608	GCCCTTGGGG[G/T]TTGCAGGCTG	8 (7)	2 (1)	3' UT
.20	651	GGGCTGGGGG[G/A]ATCCCAGCAG	8 (8)	2 (2)	3' UT
1286.20	5366	GGCCATTGCC[G/A]CAGTCGCAGC	12 (11)	2 (2)	3' UT
1287.10	864	AGGGATGTTAGACGGAATT[C/G]C	2 (2)	4 (3)	3' UT
1289.15	885	ATCATGTGGA[G/A]GGGCCAGAGG	13 (9)	2 (1)	3' UT
.22	1006	GGCATTCCAG[C/G]TGAGACACTG	21 (10)	5 (2)	3' UT
1290.7	929	CCCTCACCCC[A/G]TCACGCCTCG	3 (1)	2 (2)	3' UT
1291.5	1060	TCAACAAAAA[G/A]GGACAGGTAC	2 (1)	2 (1)	Silent
.8	2168	TAAGTACCAC[G/A]AGCAGCTGGG	2 (1)	2 (1)	Ser -> Lys
.12	4517	GCTGACAGAG[G/A]AGGAGGACTA	5 (2)	2 (1)	Ser -> Lys
.13	5114	CCAGCCTCCA[G/A]TGTACAACTT	4 (1)	2 (1)	3' UT
1292.11	3547	AGGCAAATTC[A/G]ATTTGAACAT	7 (3)	5 (3)	3' UT
.20	3888	TGTGTGTGTG[T/G]GCTGTCGCTT	11 (9)	3 (3)	3' UT
.21	3889	GTGTGTGTGT[G/T]CTGTCGCTTG	11 (9)	4 (3)	3' UT
1293.10	2480	CATGCCTGTG[C/G]GTGCGCTTCC	2 (2)	3 (2)	3' UT
.11	2481	ATGCCTGTGC[G/C]TGCGCTTCCT	4 (4)	2 (1)	3' UT
1298.20	960	TTCAGTGGGC[T/C]TTTCTGGCAG	12 (8)	2 (1)	Leu -> Pro
1300.7	566	AAGTGTACCT[T/G]GAATTCTTTG	2 (2)	4 (2)	N/D

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1301.12	668	CGCCCGGCTG[G/C]GCAAGGAGAT	9 (5)	3 (1)	Ala -> Arg
.30	1058	CAAGGTCTAT[G/C]CTGACGCCTC	16 (7)	3 (2)	Ala -> Pro
.31	1059	AAGGTCTATG[C/G]TGACGCCTCC	13 (6)	3 (2)	Ala -> Val
1302.7	759	ACAGGCCACA[T/G]CTGGACCATC	2 (2)	5 (5)	Ser -> Ala
.8	806	TATCAACTCC[C/T]GGACAACCCA	2 (2)	4 (4)	Silent
.10	866	TTCGAAGAGT[T/C]ATTGCCAAGA	4 (4)	2 (2)	Silent
.17	2000	GAATTTAATA[G/T]GTACAGAAGT	5 (5)	4 (4)	3' UT
.19	2158	ACTTCTAAAG[C/A]AAGAGGATAA	8 (7)	9 (9)	3' UT
1303.5	1226	TGCTGTGCAC[A/G]TTGACTACAA	6 (5)	2 (2)	Ile -> Val
.15	1624	GATTATATAT[T/A]TTTTTTTCTG	7 (5)	3 (3)	3' UT
.21	1813	GTGCACTAAT[A/G]TGTAAGACAA	9 (6)	3 (3)	3' UT
.22	1920	TTAAATAGCT[C/T]TTTTCTCTGA	2 (1)	14 (8)	3' UT
.23	2079	TCTATAAACC[A/G]AACTGATGTA	2 (1)	16 (9)	3' UT
1305.12	1434	AATAAACTATAGTAGTGTT[T/A]T	8 (8)	5 (4)	3' UT
1306.14	407	TTTGATATTG[C/T]CTCTGGAACT	2 (2)	4 (4)	Ala -> Val
.21	1021	TTTTTTTGCA[A/T]AAAACTAAAT	2 (2)	4 (3)	3' UT
1309.4	466	GCGGGCCGCC[T/C]GCTCTTGGAG	5 (5)	2 (1)	Leu -> Pro
.5	494	AGGAGTATGC[G/A]GCTCGGGCCC	4 (3)	3 (3)	Silent
1312.10	492	ACCCCTGGGG[G/A]AGTGCATCAT	7 (6)	3 (3)	Ser -> Lys
1315.13	339	AAGTTCCTCA[C/A]GCCCTGCTAT	13 (10)	2 (2)	Thr -> Lys
.22	766	TCCTTTTTTA[A/G]AAAAAAAAAA	8 (7)	3 (3)	3' UT
1317.4	1083	GATAGATTAT[G/A]TATTCTTCCA	3 (3)	4 (3)	N/D
1318.2	183	GGGAGCCTGC[C/A]AGGGTCCGCT	12 (11)	3 (3)	Silent
1322.12	876	TGACTCCACA[G/A]CCTCAGCCGA	23 (14)	5 (5)	Ala -> Thr
1326.5	139	GGCCTGGAAA[C/T]TTGCACAGTC	5 (5)	3 (1)	Leu -> Phe
.12	1339	TAGGAAAGAC[G/A]TCGGCTTTCG	5 (2)	3 (3)	Val -> Ile
.17	2214	TCCCCAGGGT[T/C]TTCTCATGGT	2 (2)	5 (3)	Silent
.19	2333	ATTCTGAGGG[A/G]TATCCAGCAG	4 (4)	4 (2)	Asp -> Val
1328.5	2968	CCTAAAAGTG[T/G]TTTTTATTTC	6 (4)	4 (4)	3' UT
1330.13	1526	TTGATCATGA[G/A]ACATAGGTAT	6 (3)	2 (1)	3' UT
1331.15	1666	ACAAGCACAC[C/G]TTAGAGGCTT	2 (2)	10 (4)	3' UT
.24	2009	CTGCTGATGC[C/T]GTACCCTCAC	13 (7)	2 (2)	3' UT
1332.5	618	AGCTGAACCC[G/C]GAGTCCTCCC	2 (1)	2 (1)	Silent
1333.4	89	GAGCACAGCG[G/A]CATCTTTGGC	7 (5)	2 (2)	Ala -> Asp
.10	279	CCGTGCAGGC[C/A]ATGAACCGCA	5 (5)	6 (5)	Silent
.24	756	TGACCCCCGA[C/A]CCAGCCTCGC	6 (6)	7 (6)	3' UT
1335.1	331	AGGGCTGGCC[C/T]TTGGAAGGCG	4 (4)	2 (2)	5' UT
.13	872	AGCCAAGCCG[G/T]TCAAGGCATC	7 (6)	2 (1)	Val -> Phe
.28	2268	GGAAAAGGGA[G/A]AAACTGAGCG	6 (6)	2 (2)	3' UT
1336.6	851	GCCGCGAGGC[C/G]TGGTCTGAGC	5 (5)	11 (5)	3' UT
.7	889	GGTCCTCTCA[G/A]TCTTTCCCCT	21 (10)	2 (2)	3' UT
.15	990	TTGGCAACGG[C/T]CGTCGTCATG	17 (11)	2 (1)	3' UT

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1337.12	420	GCAGTCATGC[C/G]GGGTGATCGT	32 (15)	3 (2)	3' UT
1339.17	2972	TATTAGTCCA[A/G]TGAGATTTCC	12 (9)	7 (4)	3' UT
.20	3146	GTCGGACAGT[G/T]GCTCATAGAG	6 (6)	5 (4)	3' UT
1341.3	630	CTCGTAAGGC[G/T]TCCGGTCCCC	4 (4)	6 (3)	Silent
.4	633	GTAAGGCGTC[C/T]GGTCCCCCGG	10 (9)	4 (2)	Silent
.17	896	AAAAAGGCGG[G/C]CGGAACCAAA	22 (14)	2 (1)	Silent
.29	1107	AGGCTGTGAA[G/A]CCCAAGGCCG	13 (8)	2 (1)	Silent
.32	1195	AAACCCAAAA[G/A]GCTCTTTTCA	7 (5)	5 (3)	3' UT
1342.5	142	GCGCCAAAGC[G/A]AAATCCCGCT	11 (9)	3 (2)	Silent
.7	227	CGCAGAGCGG[G/T]TTGGGGCAGG	4 (4)	5 (4)	Val -> Phe
.8	271	TGTTAGAGTA[C/T]CTGACCGCCG	11 (11)	4 (2)	Silent
.10	314	CGCGGCTCGC[G/A]ACAACAAGAA	8 (8)	2 (2)	Asp -> Asn
1343.17	514	GAACTCAAAA[G/A]GCTCTTTTCA	7 (7)	4 (4)	3' UT
1344.2	149	GAGCGCATCG[C/G]GGGAGAGGCT	2 (2)	2 (2)	Ala -> Gly
1345.3	360	GGCGCGGTGG[G/C]GTCAAGCGCA	3 (3)	3 (1)	Gly -> Ala
1346.1	2269	CAGACTGGTG[A/G]ACGAATATTC	2 (2)	2 (2)	Asn -> Asp
.2	2407	CTCTGAGACG[A/C]TGAAGACCCG	2 (2)	3 (3)	Met -> Leu
.10	3265	TGCCGGGCCT[C/T]CCTCCCGGGG	3 (3)	2 (2)	3' UT
1347.3	107	GAAGCCGAGA[C/G]GGAAAATGTC	12 (8)	4 (3)	Arg -> Gly
.5	109	AGCCGAGACG[G/A]AAAATGTCAT	2 (2)	3 (3)	Silent
.6	111	CCGAGACGGA[A/G]AATGTCATCA	16 (12)	2 (1)	Lys -> Arg
.37	994	GGTTCTTGTT[T/G]GGGCACAGCA	16 (11)	3 (3)	3' UT
.38	996	TTCTTGTTTG[G/T]GCACAGCACA	17 (11)	4 (4)	3' UT
1349.4	351	ATCGGGATCG[T/A]GTGTTCCAGT	4 (1)	9 (5)	Val -> Ser
.9	1136	GCCCTGCACG[A/G]GCCCAGGGGC	19 (13)	3 (3)	3' UT
.10	1137	CCCTGCACGA[G/A]CCCAGGGGCT	10 (6)	11 (7)	3' UT
.11	1150	CAGGGGCTGA[G/A]CGTTCCTAGG	20 (12)	2 (2)	3' UT
1350.4	188	CCAAGCGCTC[T/C]AGGGGCTTTG	4 (4)	12 (7)	Silent
.5	275	ATGGAAGAGT[T/C]GTGGAACCAA	15 (10)	2 (1)	Silent
.10	473	GGGGCTTTGC[C/T]TTTGTAACCT	9 (8)	3 (2)	Silent
.12	770	ATGGATTTGG[C/T]AATGATGGAA	5 (5)	2 (2)	Ala -> Val
1351.25	1695	GTGTGGAGAA[G/A]CCACAGGCCT	10 (7)	10 (8)	3' UT
1354.23	2233	CAACAATTTT[C/T]TATGTTAGTT	7 (6)	3 (1)	3' UT
1355.7	4296	AGCCTTCAGG[C/T]TCGGGGGGCT	2 (2)	2 (1)	Ala -> Val
.8	4778	GCGCTGATAA[C/G]GTTCATGGAA	3 (3)	3 (3)	3' UT
.10	4785	TAACGTTCAT[G/A]GAACGCGTTG	5 (5)	2 (1)	3' UT
1358.8	2515	CAGGGCGAGT[G/C]GCATGTCTGC	7 (7)	2 (2)	3' UT
.17	2629	CTTGGCATGT[G/A]ATGGCAGCTC	20 (17)	2 (2)	3' UT
1359.3	297	ATAAATACAA[G/A]AACATTGGAG	3 (2)	2 (2)	Silent
1360.12	548	TGTAAGCTGA[G/C]CCTGGTGGCC	8 (6)	2 (1)	3' UT
1361.10	4077	CTGTCTTTCC[A/G]TTTTTTCATG	14 (9)	2 (1)	3' UT
1362.9	1832	CCGCCAGGCG[G/A]ATTTTGTTCA	2 (2)	2 (2)	Silent

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.11	2248	CCTATCGGCT[C/G]TTTGCAGTGG	3 (2)	3 (3)	Leu -> Val
1363.22	2874	CCGGAATCCA[A/C]AGTGCTCTGC	2 (2)	7 (5)	3' UT
1366.3	615	CGCCCATGGC[G/A]ACCAGTACAA	7 (7)	2 (2)	Asp -> Asn
.6	722	TGTACAACTT[T/C]CCCGCAGGCG	2 (2)	8 (7)	Silent
1367.18	1851	AAAAAGTAATTCCTTAAA[C/A]AT	4 (4)	4 (3)	3' UT
1368.5	2964	TCTGAGACAC[G/A]CCCCAACATG	3 (3)	2 (2)	3' UT
1372.1	276	AGATGCTAAG[A/G]TTACCTTTCC	4 (3)	2 (2)	Ile -> Val
1373.13	3855	AATATAATAT[C/T]GACACAGTGC	4 (4)	2 (2)	3' UT
1378.12	4157	TGCTGGGGCA[T/C]GGCGGGATCC	2 (2)	2 (1)	3' UT
1383.14	1832	ATCACCACCA[C/T]GTGAGTGGTA	12 (6)	4 (3)	Silent
1385.17	3454	CAGTGCTAAT[G/A]TGTGCAAGCA	7 (5)	4 (3)	3' UT
1386.31	470	GGGTGACGGG[C/G]CCATGGGGCG	5 (5)	3 (3)	3' UT
1387.5	1385	TCGGTGCAGT[T/C]TCCACTCTTG	2 (2)	2 (2)	3' UT
.7	1678	CAGGCTCATC[C/A]TGGGAGCTTT	3 (3)	5 (3)	3' UT
.8	1900	CAGCCCTGCT[G/A]ACCATCTCAC	4 (4)	2 (2)	3' UT
.11	1967	GCCCCCTGGG[G/A]AGTTGGGGAA	17 (13)	2 (2)	3' UT
.15	2075	ATTTCTTCCT[G/T]GTGGCATTAG	18 (14)	3 (3)	3' UT
.17	2089	GCATTAGCCA[C/T]TCCCTGCCTC	22 (15)	2 (2)	3' UT
.22	2234	AAGAGAGAGAGA[A/G]AAAAAAAA	13 (10)	6 (4)	3' UT
1388.17	2799	CACAGAAGCA[G/C]CTAAACCAAG	15 (11)	4 (1)	3' UT
1395.4	327	CAATGTGTTA[T/C]GTAGTGCTTA	35 (17)	2 (1)	3' UT
1396.10	1887	GGCACGAGCC[C/T]TCCTTCTATA	3 (3)	3 (1)	3' UT
.12	1921	CCCCAGTGGG[G/A]ACTGAGTTAT	3 (3)	5 (2)	3' UT
.21	2403	TGACCAGGAC[G/C]CCTCTGGCCC	2 (2)	3 (3)	3' UT
.26	2579	AAAGGCTGAA[T/A]TGTCTGAAAA	10 (7)	3 (1)	3' UT
1397.23	6232	TATTCAGAGT[G/T]GGCTGGGCCC	3 (3)	2 (2)	3' UT
1399.2	177	CCCCCGAGGG[G/A]ATGCCAAGAT	3 (3)	2 (2)	Asp -> Asn
.10	1136	AGGGGACAGT[A/G]ATAGCCAGCA	3 (3)	4 (4)	Silent
.16	1279	CTGCTGTAAA[G/A]GCTGCAGCCT	8 (8)	2 (2)	3' UT
1401.3	71	CCAAGAATCT[G/A]CTGCGCATGA	2 (2)	3 (3)	Silent
.17	874	TTATGTTTAT[G/A]TTTATTATGT	8 (6)	6 (4)	3' UT
.19	917	TTGGAATCAA[G/A]TGTCATAAGA	8 (7)	5 (4)	3' UT
.21	1081	TCTACTTTCA[A/C]AAAAAAAAAA	2 (2)	7 (6)	3' UT
.23	1083	TACTTTCAAA[A/T]AAAAAAAAAA	2 (2)	3 (3)	3' UT
1404.12	3921	TGTTGCACAC[T/C]AGCCTTACAG	3 (3)	2 (2)	3' UT
1405.15	4823	GTCCACATGC[A/G]CTGGGCGTCT	4 (4)	12 (10)	3' UT
1406.5	4618	TGCTTTCTAG[G/C]TCAGTCCCTG	5 (3)	6 (4)	3' UT
1407.5	405	CCCAGGGGGG[G/C]AGCTCCCATT	5 (4)	2 (2)	Ser -> Gln
.9	713	TCTCTCAGAG[G/A]AAGTTTTTGG	10 (7)	2 (1)	Silent

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change	
.18	1053	GGGCAGGGAA[T/C]CCTGGAGCAC	21 (13)	2 (2)	3' UT	
.21	1144	GTGGGGTGGG[G/A]TGAGTAGGAC	2 (2)	25 (14)	3' UT	
1411.4	2009	GGCGTCAGAG[A/G]TGCTGGGTGA	6 (4)	7 (5)	3' UT	
1414.13	930	ACATACGAAC[C/T]GCCTCCTTCC	16 (13)	3 (2)	3' UT	
1415.24	1362	GTGCGATTCT[A/G]GATAAAGCCA	7 (5)	3 (3)	N/D	
.26	1442	GAGAATCCCT[G/A]GCAAAGGGAG	10 (8)	3 (3)	N/D	
1420.6	461	CAGCGGGAGC[G/T]TGAAGAAAGA	2 (2)	2 (2)	Arg -> Leu	
.8	685	TGGTGGCAGT[G/T]TGGGCTCTCA	12 (8)	2 (1)	Val -> Leu	
.9	689	GGCAGTGTGG[G/C]CTCTCAGCCA	15 (12)	2 (2)	Silent	
.16	853	GCTGGCAGCT[G/T]TGAGGCTCTA	25 (19)	2 (2)	Val -> Leu	
1421.8	169	AAGTATACAG[A/G]ACAGATTACA	20 (14)	2 (1)	Silent	
.25	1166	GTTAGTTTTC[T/C]GGCCCGTGGC	4 (3)	3 (2)	3' UT	
.26	1167	TTAGTTTTCT[G/T]GCCCGTGGCC	4 (3)	11 (7)	3' UT	
.29	1275	TCTGGCATAC[C/G]GATAGGCTTA	6 (5)	14 (11)	3' UT	
1422.7	278	CCGGGAACCG[G/C]CCACCATCAA	4 (3)	3 (3)	Ala -> Pro	
1424.3	1012	GGGAGGATGC[T/G]CTCTCTCGCG	2 (2)	5 (3)	Silent	
.4	1021	CTCTCTCTCG[C/T]GTAGCTGGAA	5 (3)	2 (1)	Silent	
.7	1295	GTTTAATGCA[T/A]GGATTCGAAA	2 (2)	3 (2)	Trp -> Arg	
1425.3	274	GCACTGGAGG[G/T]TTTAATTTTG	2 (2)	2 (2)	Gly -> Val	
1426.2	1364	GATCACCAGA[T/C]ACCAGGGTGT	9 (6)	2 (1)	Tyr -> His	
.17	2298	TCTCCAGAGT[C/T]ACTCCGTTCT	4 (4)	3 (3)	Ser -> Leu	
1427.3	90	CGCCGGCTGC[G/C]CTGCAGGTGA	8 (6)	3 (1)	Silent	
.4	91	GCCGGCTGCG[C/G]TGCAGGTGAC	8 (6)	3 (1)	Leu -> Val	
.6	109	GACAGTTCGT[G/A]ATGCTATAAA	12 (6)	2 (2)	Asp -> Asn	
.11	438	TCTTCAGGGG[A/G]CCCAATGGTG	7 (2)	2 (2)	Glu -> Gly	
.23	1172	CTATTCATAA[A/C]GGAAAACGAT	10 (5)	12 (7)	3' UT	
.24	1179	TAAAGGAAAA[C/T]GATTTCTAAA	21 (10)	2 (2)	3' UT	
.31	1323	CAAATTATAT[C/A]ACATTTTATC	8 (3)	13 (10)	3' UT	
.34	1376	GCAGAGTCCT[G/C]ATGAAAGATG	13 (7)	5 (4)	3' UT	
.37	1433	GCATATAATA[C/T]ACATTTACTG	6 (2)	9 (7)	3' UT	
1430.3	682	TCTTTGGGGA[G/A]TCAGATGAGC	7 (6)	2 (2)	Ser -> Glu	
1431.2	79	GCCAGTGGCG[C/T]TTCGTGGACG	7 (6)	2 (2)	Silent	
.6	296	TCACGCAGTG[G/C]CCAATAATCA	10 (7)	7 (6)	Ala -> Pro	
1432.8	2640	AAGTTGCTTA[G/A]AGAGCCACCA	8 (7)	2 (1)	3' UT	
.9	2695	GTTTTAATGC[A/C]AAGGAAATTT	12 (9)	3 (3)	3' UT	
1433.7	1695	AGCCGGGCTG[C/T]TACCTGCCCA	3 (3)	2 (2)	Silent	
.10	2052	CCCCTGGGTG[C/T]GGGGTGATCG	2 (2)	2 (2)	Silent	
.11	2160	ATGAGTCCAC[T/C]CTGGCCTTCC	2 (2)	2 (2)	Silent	
.23	2698	GGACCTTCGA[G/A]GGCCTCTGCC	4 (4)	3 (3)	3' UT	
.28	2787	GTGGAGGAGA[G/A]GCCTGTGGCC	6 (6)	2 (2)	3' UT	
.30	2844	GGTGGCGCAG[C/G]CTTGGTAACG	15 (13)	8 (6)	3' UT	
.31	2848	GCGCAGCCTT[G/A]GTAACGCCAT	15 (13)	8 (6)	3' UT	
.32	2857	TGGTAACGCC[A/G]TGGACTGCAG	16 (14)	8 (6)	3' UT	
.33	2877	GCGACAATCA[A/G]TGGATGGTGC	16 (14)	8 (6)	3' UT	
.34	2942	CCCTACCTGT[C/T]TTATTTCATA	17 (14)	14 (9)	3' UT	
1434.15	2041	ACTGTACCTT[C/T]TATGGTTTGC	2 (1)	5 (4)	3' UT	

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.17	2127	TGATTAGAAC[G/T]GGTAGCCAGT	2 (1)	5 (4)	3' UT
.18	2154	AATATTGATA[G/T]AAAAATAAAA	2 (1)	5 (4)	3' UT
1437.16	2825	AGTTTAAGAT[G/C]ACTTGACCCC	5 (4)	3 (2)	3' UT
.19	3129	CATGCGTAGC[C/T]TCTTGTCTTA	7 (5)	3 (2)	3' UT
1440.5	940	AACTTCAGAA[G/A]GCCAGTGTTG	2 (1)	3 (3)	Silent
.6	1327	TGGCCCTGCC[T/C]GGGAAGCCGC	2 (1)	2 (2)	Silent
.9	1906	GACCTGAAGG[C/T]GAACGTGATA	2 (1)	2 (2)	Ala -> Val
.14	2282	TCTTAGAGGC[C/T]TTTCTTGTAT	2 (2)	3 (3)	3' UT
1443.4	1943	CTTCGTGCGA[G/A]AACCTGAGAA	3 (2)	2 (1)	Glu -> Lys
1444.31	1905	CCAACAGCCT[C/T]CAAAGATGGG	3 (2)	28 (20)	3' UT
1445.4	425	CCAGGCTTGC[C/A]AGCCGAAACG	8 (5)	2 (2)	Pro -> Gln
.25	1281	AACAAAGAAA[A/T]AAAAAAAAAA	5 (4)	4 (4)	3' UT
1446.3	1227	AGGTGTGGAA[C/T]ACCCTCAGCG	2 (1)	2 (2)	Silent
.17	3090	TTATTTATAT[T/C]TTTAACATAA	10 (7)	2 (2)	3' UT
1447.8	2681	GGCAATAGCA[A/G]TCTTGGCTGA	3 (3)	3 (2)	3' UT
1448.2	521	AGAAGACCAC[A/G]ATGCGAGATG	3 (2)	3 (1)	Silent
.3	587	GTCATGCTCT[T/C]GCACTTTACA	4 (3)	3 (1)	Silent
1449.20	1261	TGCGTAATGC[G/A]GCCGAAGAGC	4 (3)	21 (13)	Silent
.28	1447	CTGAGAGCCC[C/G]AGGCGTCCGC	21 (14)	2 (1)	3' UT
.31	1652	TTGCAGATTG[A/C]ATAAAAAAAA	8 (6)	6 (4)	3' UT
.32	1653	TGCAGATTGA[A/T]TAAAAAAAAA	11 (7)	3 (3)	3' UT
.33	1654	GCAGATTGAA[T/A]AAAAAAAAAA	6 (6)	4 (4)	3' UT
1450.2	156	CCCCATGGCG[G/A]CCGCCAAGGA	11 (9)	2 (2)	Ala -> Thr
1451.13	200	GATGAGCGTG[A/T]TTCCTCTCGA	3 (2)	31 (20)	Asp -> Val
.14	201	ATGAGCGTGA[T/A]TCCTCTCGAT	3 (2)	31 (20)	Asp -> Glu
.18	417	AAGTTCACAT[C/G]AACCTCATGG	2 (1)	28 (18)	3' UT
1452.12	1659	GTACCAGAGG[C/T]ATGCCTATCA	4 (4)	2 (1)	Ala -> Val
.18	2410	ATTTAAGGAC[G/A]AGACCAGCAG	3 (3)	9 (5)	Silent
.19	2419	CGAGACCAGC[A/G]GCTAATCCAA	9 (8)	3 (1)	Silent
.23	2717	GTTAATGATG[T/A]TAATGATTTT	17 (13)	5 (3)	3' UT
1454.3	338	AGGGCTTTGC[C/T]TTCGTTCAGT	3 (2)	6 (2)	Silent
.7	1211	CATGCTCACT[G/T]TTCTCCCCAT	9 (6)	2 (1)	3' UT
.8	1391	GTTTTTAAAAAAA[A/T]AAAAAAA	3 (2)	3 (3)	3' UT
1455.6	294	CCAGGCCTTT[G/T]TCATCTTCAA	9 (8)	2 (2)	Val -> Phe
.22	911	CAGCTCGCGA[T/A]GCCCTGCAGG	13 (12)	3 (3)	Asp -> Glu
.23	912	AGCTCGCGAT[G/T]CCCTGCAGGG	8 (8)	4 (4)	Ala -> Ser
1460.1	6	AATTC[C/G]CAGAGCAACATGCCC	5 (5)	3 (3)	5' UT
.30	547	GTTCTGCTTC[A/C]CCAGGAGATC	25 (17)	5 (3)	3' UT
1461.5	154	TCCCCGGGGG[G/C]CTTTGGATCG	8 (7)	2 (2)	Silent
.32	1463	GTGTTACTGC[A/G]TTTTGTACAA	14 (8)	11 (8)	3' UT
1463.3	761	CAGCGTGGGG[G/T]TGGCCACTCC	2 (1)	2 (2)	3' UT
1464.3	21	GCCTGCAGGC[C/T]TCCCGAGGAG	6 (3)	2 (2)	Silent

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.4	130	GCAGACTTAT[A/G]AGGTTGACCT	3 (1)	11 (7)	Lys -> Ser
.5	132	AGACTTATAA[G/A]GTTGACCTTA	3 (1)	10 (7)	Silent
1465.4	897	AGTTCCACCC[T/C]ACAGGCATAT	2 (2)	3 (3)	Silent
.5	1044	TGTCTCGGTC[C/G]ATGACTCTGG	4 (4)	2 (2)	Silent
.12	1758	GAGCAGAGGC[A/G]CGGAAGGAGT	8 (8)	3 (3)	Silent
.30	1892	ACCCTGTCCT[A/T]TGTGGACGTT	19 (17)	6 (6)	Tyr -> Phe
.34	1938	ATAGACCCGT[G/A]ATCGACAAAA	16 (15)	9 (9)	Silent
.37	1975	CTGTGCCACC[G/A]TCCCGCCAGC	21 (20)	6 (6)	Val -> Ile
.38	1980	CCACCGTCCC[G/C]CCAGCCATTC	21 (20)	5 (5)	Silent
.41	2014	AGACAAGATG[T/C]GGTGATGACA	22 (20)	5 (5)	3' UT
.42	2102	TTCTGCACTC[T/C]GGGGAAGAAG	23 (20)	8 (7)	3' UT
.45	2139	GATTGGCACC[T/C]AGTGGCTGGG	24 (20)	7 (6)	3' UT
1467.9	2297	CATGGAGGCA[G/A]CCAGGCCCGT	4 (4)	2 (2)	Ser -> Asn
.11	2353	TAATAATATG[T/C]ATGCCTGGGG	3 (3)	2 (2)	Tyr -> His
1471.4	3042	CACCCAACCT[G/A]TCCTTACTCA	2 (2)	3 (1)	3' UT
1473.9	390	GAAAAGCTGC[C/T]ATTCTCAAGG	13 (11)	5 (3)	Silent
.10	399	CCATTCTCAA[G/A]GCCCAAGTGG	11 (8)	3 (3)	Silent
1474.1	8	TCT[G/A]AACGGAGAGCGTAGTGA	13 (10)	4 (3)	5' UT
.2	9	CT[A/T]ACGGAGAGCGTAGTGACC	14 (11)	3 (3)	5' UT
.9	94	GCGAGAGGAG[G/T]AGGAATTTAA	27 (14)	2 (1)	Ser -> ***
.24	370	GCGGAACCCG[C/T]TCATCGCCGG	21 (15)	3 (2)	Leu -> Phe
.26	392	AAGTAGGGGC[C/A]GCCTGTCTGT	28 (14)	2 (1)	3' UT
1476.6	230	CACAAGTGCC[C/T]TTCGAGCAGA	12 (9)	2 (2)	Silent
1477.20	1470	ATTTGATGGA[G/C]GCTGCGCCGG	31 (12)	6 (4)	Ser -> Asp
.24	1480	GGCTGCGCCG[G/C]AGTGAAGAGG	34 (14)	2 (2)	Ser -> Gln
.28	1647	TTCCTGTTGA[A/T]AAAAAAAAAA	9 (6)	3 (2)	3' UT
1478.19	838	TATGGAAGTA[G/A]CTCCGCAGAG	17 (11)	2 (2)	Ala -> Thr
.29	1009	TCCTCAGCTC[C/T]CTGCCTGTTT	26 (18)	2 (1)	3' UT
.30	1095	AATAAACTCTTAAAGA[G/A]CCTT	2 (2)	24 (16)	3' UT
1480.17	913	AAGAGGCACT[G/T]TAGCAGCTGC	17 (13)	2 (2)	Val -> Leu
.18	939	TTGCTGCGAC[T/C]GCCAGTATTG	18 (13)	2 (2)	Silent
.19	979	CCCACCAGGA[C/A]GGGGCACTCC	17 (12)	4 (4)	Silent
.20	980	CCACCAGGAC[G/C]GGGCACTCCG	11 (10)	4 (4)	Arg -> Pro
.29	1113	TAGGCATGCC[G/C]CCTCCGGGAA	20 (13)	2 (2)	Silent
1483.12	1969	ACTTCTCCAT[C/T]CGGTCCCTAG	2 (1)	2 (2)	Silent
1484.2	140	ATTACGATGA[G/A]GAGGAAGAGC	3 (2)	12 (8)	Ser -> Glu
.7	288	CTGTGGCTTG[G/A]AGCATCCTTC	8 (7)	2 (2)	Ser -> Lys
.11	674	AGCACTTTGT[G/C]CTGGACGAGT	3 (3)	2 (2)	Silent
1486.24	6427	GCATTAACTA[A/T]AAAAAAAAAA	5 (5)	7 (5)	3' UT
1487.15	2896	GCGCCAAGCC[C/A]AGCAGGCTAC	3 (3)	3 (1)	Pro -> Gln
.20	3303	AGCCACGGGC[G/T]TCCTACTGAG	8 (7)	3 (3)	Val -> Phe
.22	3394	CTGGGGAAGC[T/C]CCTGGAAGCC	11 (10)	2 (2)	Leu -> Pro
1489.14	1419	ACTCAACTCA[C/A]GGTACAAGAC	7 (5)	3 (3)	3' UT
1490.6	443	AGGCTGCTCG[T/C]GTTGCTATTG	2 (2)	2 (2)	Val -> Ala
.31	1710	CTCGTGATGC[A/G]TCTACAGTTA	11 (7)	19 (12)	3' UT

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.33	1824	GTGGGGGTAC[C/T]ATCTCAACTG	7 (4)	13 (9)	3' UT
1491.21	1488	GCATATGGGA[G/C]CCATTGGCTG	11 (8)	2 (2)	Ser -> Asp
.31	1826	TGTAAGGTTT[C/T]CATTTAGTTT	28 (16)	3 (1)	3' UT
1495.3	391	CAAAAACCCC[G/A]CCCGCTCCAA	3 (2)	3 (2)	Silent
1496.5	3017	AATAATAACC[A/G]AGACTTTTCA	6 (4)	2 (2)	3' UT
.15	3932	CTGCCTGGCC[C/T]TTTTTTCTTC	3 (1)	6 (5)	3' UT
1497.13	1332	GCCCCATGTC[G/A]CTGGGTGGGC	3 (2)	5 (5)	Silent
.14	1338	TGTCGCTGGG[T/C]GGGCGGCACG	3 (2)	5 (5)	Val -> Ala
.16	1508	GCCACGGCGG[C/T]CGCCAGCGAG	8 (4)	2 (2)	Ala -> Val
.20	1608	CCCCCCGGGC[C/G]CGGACCAGCC	6 (4)	5 (3)	Silent
.23	1713	AGCGGCTGCG[G/T]GTCCGTGACA	6 (3)	3 (2)	Silent
.39	4022	GGCTTCCCCT[G/A]CGCCCTGGGA	3 (2)	6 (5)	3' UT
.43	4187	AAACAGCAGT[T/C]CCTGGGAACC	12 (10)	2 (1)	3' UT
.44	4254	TTTCAAAAAA[T/A]TTTTTTTAAA	2 (2)	11 (9)	3' UT
1498.5	167	GGCGTGCTGA[G/C]TGCCCTGGGA	8 (4)	3 (3)	Ser -> Thr
1500.16	2206	GAAGGAAACA[G/A]TGCAACAGCA	16 (13)	2 (2)	3' UT
.18	2310	GTTGTTAAGA[G/T]TGGGGGAGAG	25 (18)	2 (1)	3' UT
.23	2426	TGCCAAGCTG[G/A]ACGGCACGAG	10 (7)	4 (4)	3' UT
1501.5	388	GCGCTGTGCG[G/T]TGTCCCCGTC	2 (2)	2 (2)	Silent
.16	1238	CCCCGGGAGG[G/A]AGCTGACTGA	8 (8)	2 (2)	3' UT
1505.9	3934	TTAGTCATTC[T/C]AAAAAACACC	6 (4)	4 (4)	3' UT
1507.2	130	CCCCGAGGCG[A/T]TCGTGGAGGA	3 (3)	3 (2)	Ile -> Phe
1508.19	5111	CATCGCCGAG[G/C]CCTGGGCCCG	12 (10)	3 (2)	N/D
1510.6	1066	CAAAGGAGCT[T/C]GAAGGATATT	2 (2)	5 (5)	3' UT
.8	1136	TCTAAAAGAA[A/G]AAGGAACTAG	3 (2)	2 (1)	3' UT
1511.10	222	CTACAATATT[C/G]AAAAGGAGTC	18 (11)	2 (1)	Gln -> Glu
1514.6	103	CGGGGCTGCG[G/A]CCGCCCGAGG	11 (5)	4 (4)	5' UT
.24	624	GGCATCGTCA[G/A]AAGGAAGGGA	13 (5)	6 (5)	3' UT
.35	879	GCTGTAAAAT[T/C]ATAAACTTTT	27 (12)	2 (1)	3' UT
.38	913	TCCCCCAGGG[G/C]CGAGTTCCTC	25 (11)	3 (2)	3' UT
.39	914	CCCCCAGGGG[C/G]GAGTTCCTCG	20 (11)	3 (3)	3' UT
.43	1069	AGACCCCAGG[G/T]CAGCATCTCG	21 (9)	5 (4)	3' UT
1515.6	175	CATGCTAGCA[T/G]GGCCTAATGA	3 (2)	9 (8)	Trp -> Gly
.28	855	CTGGAGAGCT[T/G]GGCTTCCGCG	15 (11)	4 (4)	Silent
.30	858	GAGAGCTTGG[C/G]TTCCGCGCTT	6 (6)	7 (5)	Ala -> Gly
.38	1146	ATAATAAAAG[T/A]TTCATTTGCA	2 (2)	23 (14)	3' UT
1517.9	742	AATCATAATG[G/C]TTCTCCCCTT	6 (3)	2 (2)	Val -> Ala
.16	1424	AAGTTATTGG[C/T]AAACGAGGTT	11 (7)	3 (3)	Ala -> Val
1518.8	947	AGAGCTGAGC[G/A]AGTTCACCAC	5 (4)	2 (2)	Ser -> Lys
1519.15	1209	CCATCAAAAG[C/T]TTTGAGAATT	2 (2)	6 (5)	Silent
1520.12	6696	CAGCCTCATC[G/A]ATCCCAAAAC	5 (2)	3 (1)	Asp -> Asn
.13	6806	TGCGCGGGAG[C/A]AAACTGCTCT	2 (1)	3 (1)	Ser -> Arg

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1521.6	851	AGACTCTGAG[G/C]CCTGGTGTGA	7 (6)	2 (2)	Arg -> Ser
.10	976	TTGGGAATGG[A/G]TATCAGAAGA	15 (8)	4 (1)	3' UT
.15	1165	TCACCTATAC[A/G]TTATTTAAAT	20 (8)	4 (1)	3' UT
.17	1236	GAAAACTGTG[C/A]AATTGTGTGC	7 (4)	3 (1)	3' UT
1523.7	417	CACCACGGTG[C/T]TGGAATTGTT	9 (8)	3 (3)	Silent
1524.13	2996	AAAATGACAT[T/G]AGTTTGAAAA	3 (2)	3 (2)	3' UT
.22	3384	AACAGCTTTT[A/T]GGCCAAGCTG	20 (9)	4 (4)	3' UT
.23	3385	ACAGCTTTTA[G/A]GCCAAGCTGG	16 (7)	6 (5)	3' UT
.25	3397	CCAAGCTGGC[C/T]TGACGGTATG	25 (11)	4 (3)	3' UT
.26	3398	CAAGCTGGCC[T/G]GACGGTATGG	25 (11)	3 (2)	3' UT
1526.6	2476	TGGAGGTGCA[T/C]AACCTACTTA	2 (1)	2 (1)	Silent
.7	2715	GTGAAAGGGG[A/C]CGTGTACTCT	2 (2)	3 (1)	Asp -> Ala
1528.6	770	CCAAAAGGAA[G/A]TGAATCAGCA	2 (2)	2 (2)	Val -> Met
.10	2396	GCAGTGCGCA[A/T]TCCTGGACCT	1 (1)	4 (4)	Val -> Phe
.26	3317	TTCAAGTGAA[G/C]ATGCTGAAAG	12 (8)	7 (6)	Asp -> His
.32	3598	TATAATTAGT[T/C]ATGACAGCCA	19 (16)	2 (1)	3' UT
1530.8	427	ATCCGCCCCC[A/G]CGACGTCCCC	4 (3)	2 (1)	Thr -> Ala
.13	894	TGCTGAACGA[G/A]CCCCCTGGGG	8 (5)	2 (1)	Ser -> Glu
.30	1579	AGTCCTGAAA[G/A]GCCCAAGGCC	4 (3)	7 (6)	3' UT
1532.6	496	TCGTGCGCAA[C/T]GTGCCCTGGG	4 (2)	6 (3)	Silent
.10	963	CTGGCCTTAT[G/T]CCCAGGCCTG	6 (4)	2 (2)	Cys -> Phe
1533.12	2092	GTATCCCAGG[A/G]CACACAGGAA	3 (3)	2 (2)	Asp -> Ala
1534.4	264	CCGTGCCGGC[A/T]CTTCACCATC	2 (1)	5 (4)	Silent
1536.22	6641	TTAGATATAT[A/G]TATTCATTCT	3 (3)	4 (3)	3' UT
.24	6779	ATTTTTATTG[G/A]GCCCAAAAAC	2 (2)	11 (8)	3' UT
.28	7097	AGTGGAATGT[T/A]TAAAAAAAAA	4 (3)	4 (3)	3' UT
1537.5	871	AGGGCAGTGC[C/A]ATTGATAGGA	7 (6)	3 (3)	Silent
.10	1466	GCAGGCATGC[C/A]AGTCTCTGCC	7 (7)	3 (3)	3' UT
1538.21	938	CCTCCACCTT[T/C]GACGCTGGGG	14 (7)	3 (2)	Silent
1539.1	67	TCGCGGCCTA[G/C]CTTTACCCGC	3 (3)	2 (1)	5' UT
.3	304	TCGATGGCTC[T/C]AGTACTTTAC	4 (4)	4 (3)	Silent
.9	1075	GTAGCGCCAG[A/C]CTACGCATTC	2 (2)	3 (2)	Arg -> Ser
.16	2048	CAAGGAAGTG[G/A]TTCTTAGATG	8 (7)	4 (2)	3' UT
.21	2718	GCCTAACATAA[A/G]AAAAAAAAA	8 (8)	3 (3)	3' UT
1541.1	4123	TGGCGAGGGG[G/C]CTTGACGGCG	2 (1)	2 (2)	3' UT
1543.4	319	GCACCGGAAG[G/A]AGGCGCTGAC	6 (5)	2 (2)	Ser -> Lys
1544.3	534	TTGAGCCCAA[C/G]TGCTTGGACG	2 (2)	7 (4)	Asn -> Lys
.4	543	ACTGCTTGGA[C/T]GCCTTCCCAA	4 (4)	7 (4)	Silent
.8	643	ACCTGTGTTC[T/A]CAAAGATGGC	12 (8)	3 (3)	Ser -> Thr
.12	728	GCTGCCCAGG[C/G]TGTGCAGCGC	12 (11)	4 (1)	3' UT
.21	902	AACATCCCCT[C/T]CCATCATTAC	5 (4)	4 (2)	3' UT
.22	986	CTGCCTGGCC[C/T]CTGCCTGTG	5 (4)	2 (2)	3' UT
1545.4	1470	CGGTGAGACC[G/A]TTGCCCGCTG	2 (1)	2 (2)	Val -> Ile
1546.1	172	CTCTGAAGAC[A/T]TGGAGATACT	3 (1)	3 (3)	Met -> Leu

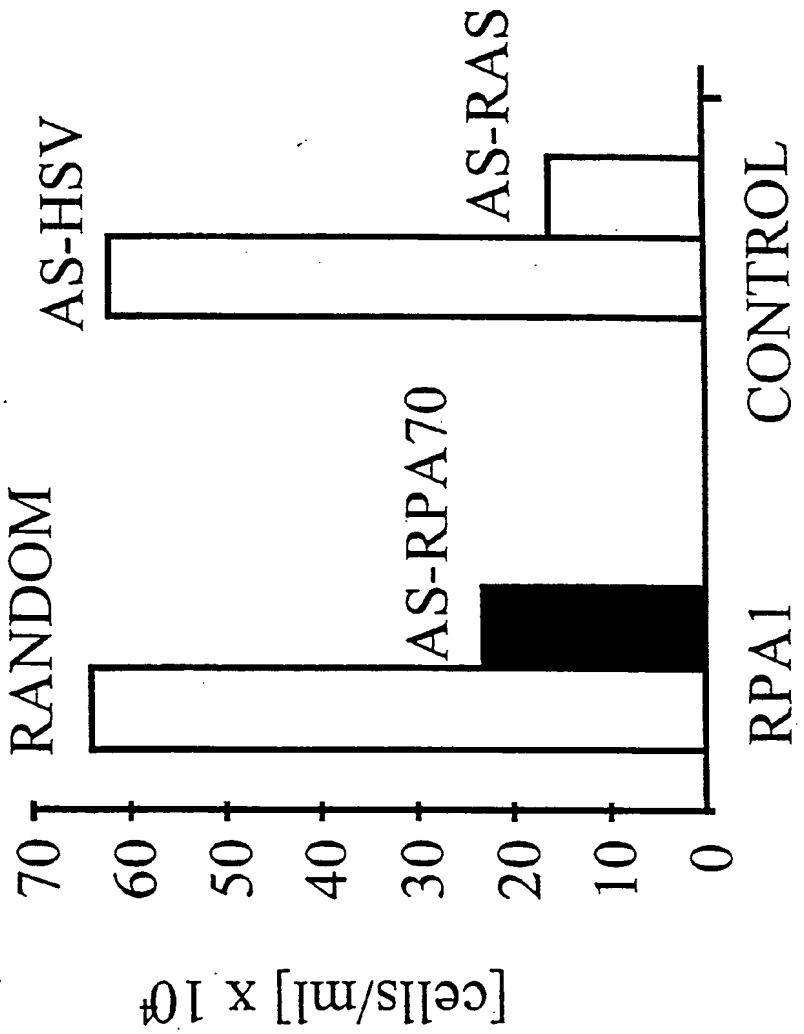
Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1547.17	976	TGCTTTAAAG[G/A]GCCTGCCTGG	13 (10)	2 (2)	3' UT
1548.3	1209	CATTATTGGC[C/T]TCATCAAACC	3 (3)	3 (1)	Leu -> Phe
.4	1286	TGAAAGGTGT[A/G]AATAAGTTAC	2 (2)	3 (2)	Silent
.8	1904	ATAACTAAGA[C/T]TTCTGTGCAT	6 (3)	5 (3)	3' UT
1550.7	797	TGGACGCCTT[T/C]CCAAATCTGA	2 (2)	5 (2)	Silent
1551.12	2215	CGAGACCATC[T/C]TGGCCCCTCC	3 (1)	10 (9)	3' UT
.14	2242	TGCCTGAGCC[T/C]AGGAGCTTGA	3 (1)	9 (8)	3' UT
.15	2341	ACTGGGTCTC[G/A]CTCCGAGTGG	3 (1)	9 (8)	3' UT
.16	2372	GGAGGGAGGG[T/A]CAGGGGGAGG	3 (1)	9 (8)	3' UT
1554.12	834	GGGACTTTAT[C/G]GATTGCTTCC	6 (5)	2 (1)	Ile -> Met
.14	999	ACCCAGAGGT[C/G]ACAGCTAAAG	8 (6)	2 (1)	Silent
.23	1539	ATCTGGCTGC[T/C]GATCTGCTAT	5 (4)	5 (4)	3' UT
1555.5	424	TATGGATGCC[A/G]AGCACCACAA	17 (8)	3 (1)	Lys -> Ser
.9	515	GCCAGCACCA[G/C]CCAGGAGCTG	17 (7)	3 (3)	Ser -> Thr
.30	1088	TCCTCGGCTG[C/A]GTTCAGTCCT	2 (2)	8 (5)	3' UT
1556.7	2037	TGATCTTTGC[C/T]CCTGGTATGC	5 (5)	5 (3)	3' UT
1560.7	2335	GCATTCAAGA[C/T]GGATACAGAG	5 (5)	2 (1)	Thr -> Met
1561.1	90	CTGTGCTGCC[C/T]GGCTCCCCCA	2 (2)	2 (2)	Silent
.5	373	CCCTGACATC[A/G]TGGAGTTCTG	2 (1)	2 (2)	Met -> Val
.22	1250	TGTTTCCTTT[T/G]GGGCTCAAAG	8 (7)	4 (4)	3' UT
.23	1251	GTTTCCTTTT[G/T]GGCTCAAAGC	7 (6)	4 (4)	3' UT
1562.14	540	ATTGTCGCAC[C/T]TCCTACACCT	21 (9)	2 (1)	Silent
.30	799	AGCCATGAGT[G/T]GGGCTGGGCC	14 (7)	3 (3)	Gly -> Trp
1563.10	3076	ACTCCCCTTC[A/G]TGAAACCAGA	2 (1)	2 (2)	Met -> Val
1564.7	339	CTTTGGAAAG[T/C]GTGAAAGCTG	15 (10)	2 (1)	Silent
1566.2	53	GCAGGCACAG[T/C]GTCACCTTCG	2 (1)	2 (2)	5' UT
.4	175	TCCTGGCGGC[G/A]CCTCGTGTGC	3 (1)	4 (4)	Arg -> His
.10	791	GCATGAATCC[C/T]GGCCCAGGCG	3 (1)	4 (4)	Silent
.23	1741	TGCACTCTGT[G/C]CTCCGCCCAA	3 (2)	3 (2)	Cys -> Ser
.24	1742	GCACTCTGTG[C/G]TCCGCCCAAG	3 (2)	3 (2)	Cys -> Trp
1567.2	1083	GGAATACTGG[G/A]AGAATCTTCG	5 (3)	2 (1)	Ser -> Lys
1571.4	1480	AGAGAAAATT[G/A]GGGAAAAGGT	4 (4)	3 (2)	3' UT
.14	2087	TCTGTCTGGT[G/A]TGGTATGAAT	5 (5)	4 (2)	3' UT
1576.13	1777	CGCCCCTCCC[C/T]CCTCTGGCCC	3 (2)	2 (2)	3' UT
.16	2031	AATTGTACATTC[C/T]CTGCATCC	3 (2)	2 (2)	3' UT
1577.10	3022	TGCCGGCCGG[A/G]ACCCAGCGGC	2 (2)	6 (5)	Asn -> Asp
.15	3229	CACACCACCG[T/C]CCTCCTCGCT	2 (2)	5 (4)	3' UT
.33	3859	GGTAGCCACC[G/A]CCGGGGCACT	18 (13)	4 (3)	3' UT
.38	3980	CTGATGCATC[G/A]TTTTCTTTGC	18 (14)	4 (3)	3' UT
.47	4049	GCCAGGCCAT[G/T]GCCAAGGGGC	7 (6)	3 (3)	3' UT
.50	4055	CCATGGCCAA[G/A]GGGCCAGCTG	5 (5)	5 (5)	3' UT
1578.5	178	TACTTCGACC[G/A]CAAAAGACGA	7 (7)	2 (2)	Arg -> His
.12	451	CTTCCACCAC[C/T]AGTGTTCCAG	8 (6)	3 (2)	Pro -> Leu

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
.13	468	CCAGATGCTT[C/T]TGACTAAGCT	8 (6)	3 (2)	Silent
.15	501	TCAGAGAATT[G/C]TAAGTGCTCA	5 (5)	2 (2)	Val -> Leu
.17	551	AAACAAATGT[C/T]AACATAATAA	5 (5)	4 (3)	3' UT
.19	630	GGGCAAATAT[G/C]CTTGTTATGA	7 (6)	2 (2)	3' UT
.20	683	CTTTGTGTAG[A/G]TCCATTTGTC	9 (7)	2 (2)	3' UT
.25	2725	AGGTGAGAAC[A/G]AAAAAACCCC	6 (5)	3 (3)	3' UT
1579.15	1735	GCTGCAGCGG[C/T]TGGCAGACGG	17 (12)	2 (2)	Silent
.19	1881	GGATCCGAGA[G/A]GGCATGGCCG	14 (12)	5 (5)	Ser -> Glu
.26	2010	GAATACTCCC[G/C]GCCAGGGCCT	12 (10)	17 (10)	3' UT
1581.2	1897	CCGCTAAAAT[G/A]AGAATAAGGT	3 (3)	5 (4)	Met -> Ile
.5	2232	TGAATGTAAC[T/C]GCTTTAAGAA	3 (3)	5 (5)	3' UT
1583.7	1482	AAGACACAGA[A/T]GGAGGGCCCA	5 (5)	3 (2)	Glu -> Asp
.11	1772	GCTTTTAATA[G/C]TGTCATAAAG	3 (3)	2 (2)	3' UT
1584.18	576	CGCCCTCACA[G/A]CCTCCTTCTG	2 (2)	2 (2)	Silent
.34	1098	ATATGGATGG[C/T]GGTACCTTCA	3 (3)	2 (2)	Ala -> Val
.46	1708	GAGAAACCCC[C/T]GGGGACCATG	3 (3)	2 (2)	3' UT
.50	1848	GAGGGATTGA[G/A]CACAGGCACA	2 (2)	6 (6)	3' UT
.51	1857	AGCACAGGCA[C/A]AGAGGTGCTG	2 (2)	6 (6)	3' UT
1587.11	1330	GCCTGCGTGG[G/C]AACTCATGCA	7 (2)	11 (10)	Glu -> Gln
.12	1356	TCCAGAACCC[C/T]GACTTCCCAC	18 (14)	2 (2)	Silent
1588.26	1956	TTGTACACAA[T/C]CTCATTTCAT	7 (6)	4 (3)	3' UT
1590.2	172	TGCACGCAGC[C/A]ATGGCTGACA	6 (3)	2 (2)	Silent
.7	547	CGCTGGATAA[C/T]GCCTACATGG	8 (4)	2 (2)	Silent
.9	850	TCATCCGCAA[G/A]GCATCTGATG	4 (2)	2 (2)	Silent
.33	2139	AGCCGACTCT[G/T]GCCCTGGCCC	14 (9)	4 (4)	3' UT
1594.10	1730	ACCCCAGTGG[G/A]AACTGTGCAA	6 (5)	2 (2)	3' UT
.13	1975	GAAACTAACT[C/T]GGTGGCCCCA	6 (5)	9 (6)	3' UT
.14	1985	CGGTGGCCCC[A/G]ACAGGTCTTC	6 (5)	9 (6)	3' UT
1596.3	1773	TGATGTGGTA[C/T]GTCCCTCCAC	10 (7)	3 (2)	3' UT
.6	1844	GTATTCACCA[A/C]GCATTTTAGG	10 (8)	4 (3)	3' UT
.11	1899	GCATTTACAA[G/A]GCACTGCCAA	17 (12)	3 (3)	3' UT
.12	1900	CATTTACAAG[G/T]CACTGCCAAA	19 (12)	2 (2)	3' UT
.16	1949	AGAGGACCTG[C/T]GGGCTTAGAT	24 (16)	2 (1)	3' UT
1598.3	2042	ATGCCTAAGA[C/A]CAACTGCGTT	2 (2)	3 (1)	3' UT
1603.5	592	TCTGTGGCAC[T/C]GATATGACCA	2 (2)	2 (2)	5' UT
.16	2566	TGAAACTGAG[G/C]CCCATCCTCA	17 (12)	2 (2)	Arg -> Ser
.18	2662	CCGGGGAAGC[T/G]GCCGTCTAAA	13 (11)	3 (3)	Silent
.28	2953	TTAGAATTTT[C/T]CTAAAAATAA	26 (18)	2 (1)	3' UT
1605.14	2879	AACACGGCCC[T/C]GCTGTCGCTG	2 (2)	2 (1)	Leu -> Pro
.30	4011	AATTTAAAGT[A/C]TTCTCCTCCC	4 (2)	6 (6)	3' UT
1607.13	2354	CTTTCTCTGG[C/T]CCTGTCCATG	9 (8)	2 (2)	3' UT
1608.3	2120	CAGCCGCCAT[T/C]TGCAAGGAAG	2 (2)	2 (2)	3' UT
.11	2552	CAAAAGATGA[G/T]TCCTTGCTTC	16 (9)	4 (3)	3' UT
.17	2733	TCCTAAGCAG[T/C]CCTGGCTTTT	25 (11)	3 (3)	3' UT
.01	2091	CTCCTTCCAA[C/T]CCCACTCCCC	65(36)	7(7)	3'UT
.02	2120	CAGCCGCCAT[T/C]TGCAAGGAAG	25(18)	47(40)	3'UT
.04	2578	GAAATAAAAG[T/G]AGCCCAGCTG	26(19)	46(29)	3'UT
.05	969	AACCTAGTGC[G/A]ACCAAGGGAA	69(36)	3(3)	Silent

.06	2174	CCTCTCCCAG[C/T]GGCCTCCCCC	71(36)	1(1)	Silent
.07	2129	TTTGCAAGGA[A/G]GGCCTAATCA	66(36)	6(6)	Silent
1611.20	1388	AACACTGGTGCCAACCAA[G/A]AC	3 (3)	3 (3)	3' UT

Target ID	Loc'n	Sequence around [polymorphism]	# Varia 1 (Lib)	# Varia 2 (Lib)	Protein Change
1613.2	350	AGTGGCCATG[G/A]TTGGGTCAGC	10 (7)	3 (3)	Val -> Ile
.11	842	TGATCATCAT[T/C]TCCTTGCGGA	3 (3)	6 (4)	3' UT
1614.5	1343	CCTATCTGGA[T/C]ACATTTGGCC	2 (2)	3 (3)	Silent
.13	1841	CGGCGGTGGA[G/A]GCTGAGCGCC	10 (9)	2 (2)	Ser -> Glu
.23	2158	TTTTTTTTTT[T/A]AAAAAAGAAA	7 (7)	8 (5)	3' UT
.28	2261	CTGAAGTCTA[G/A]GATATTTTTC	5 (5)	2 (2)	3' UT
1615.25	2113	CCTGGCCATC[T/C]TGGGCAGTGT	16 (11)	7 (5)	Silent

Fig. 9


[cells/ml] x 10⁴
70
60
50
40
30
20
10
0
RANDOM
AS-HSV
AS-RPA70
AS-RAS
CONTROL
RPA1

Variance Specific Inhibition of mRNA levels by Oligonucleotides Against RPA1

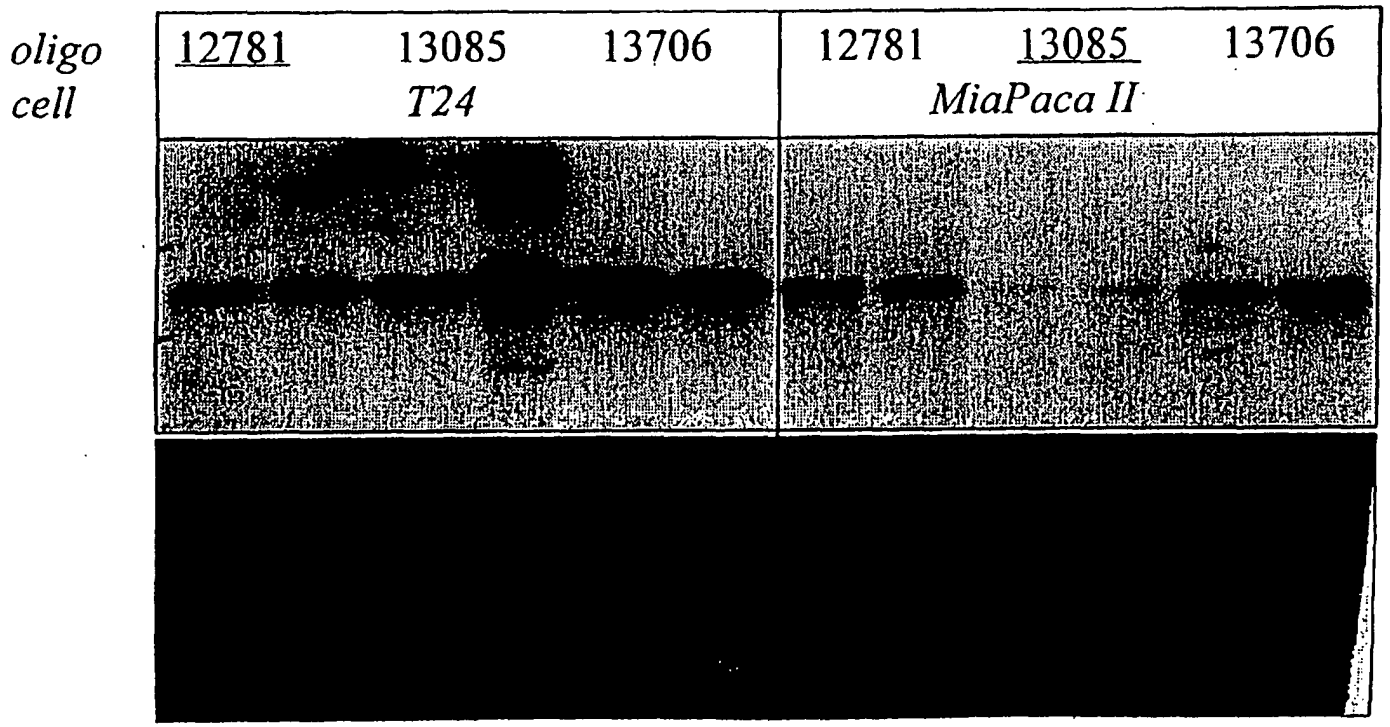


Fig. 10

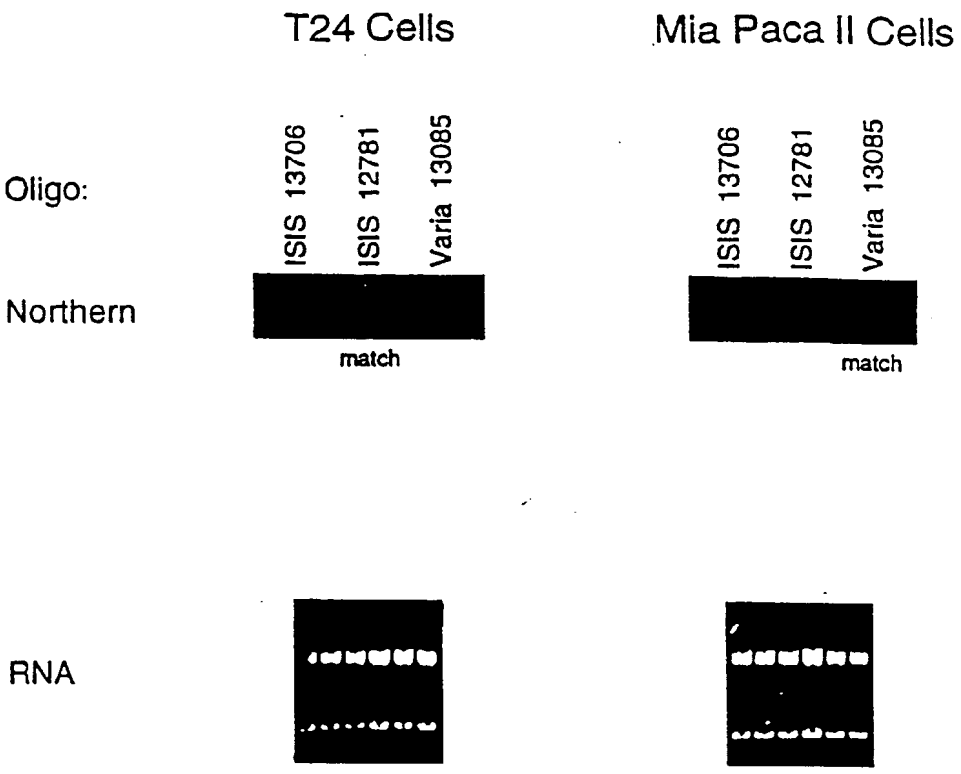
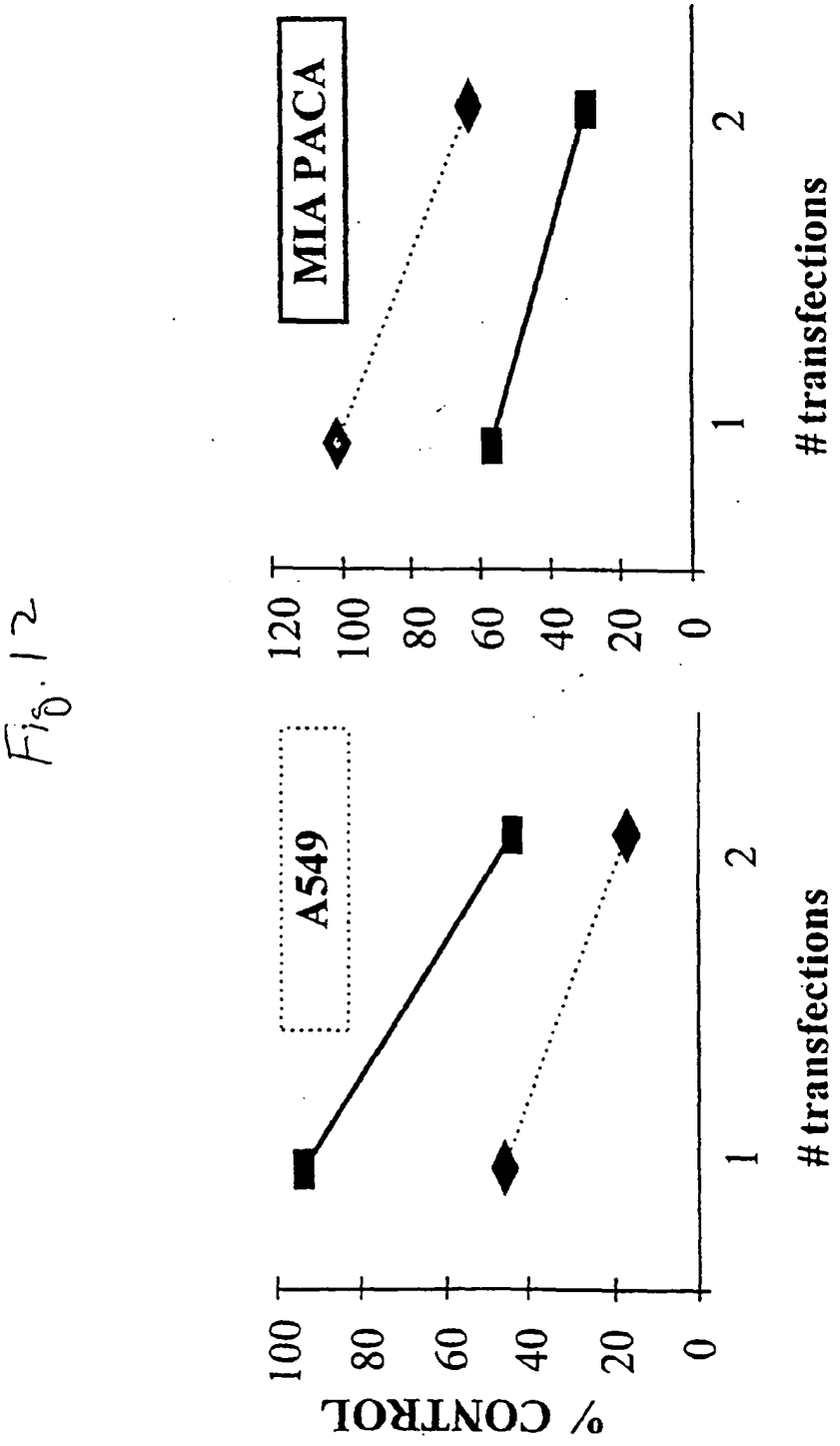

T24 Cells
Mia Paca II Cells
Oligo:
ISIS 13706
ISIS 12781
Varia 13085
ISIS 13706
ISIS 12781
Varia 13085
Northern
match
match
RNA

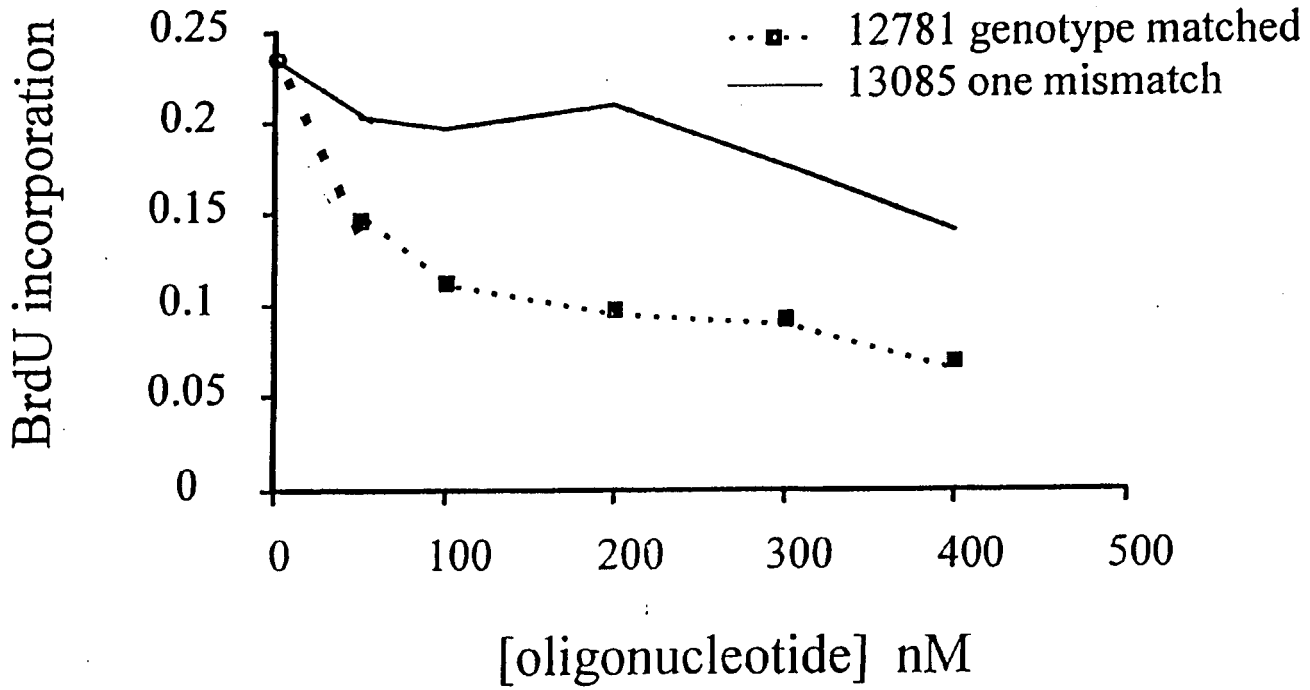
Fig. 11

Fig. 12


% CONTROL
100
80
60
40
20
0
A549
1
2
transfections
MIA PACA
120
100
80
60
40
20
0
1
2
transfections
12781 .....
13085 ——

Variance Specific Inhibition of A549 Cell Proliferation by Oligonucleotides Against RPA1

Fig. 13



Variance Specific Cell Killing of A549 Cells by Oligonucleotides Against RPA1

Fig. 14

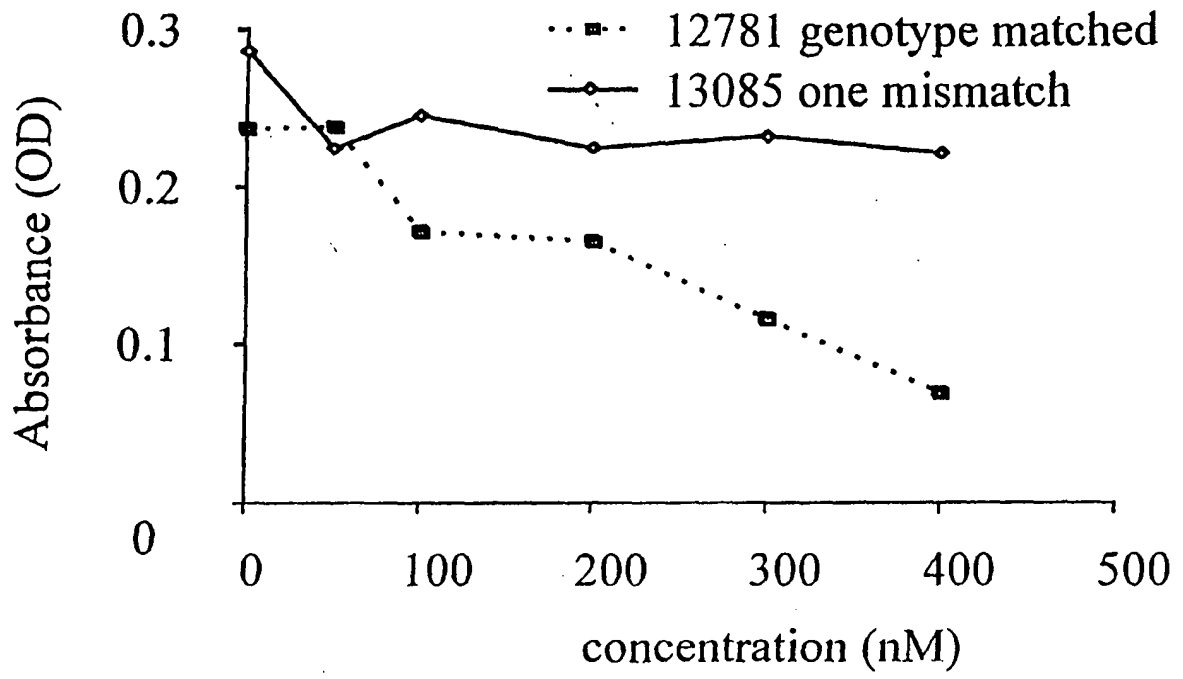


Fig. 15

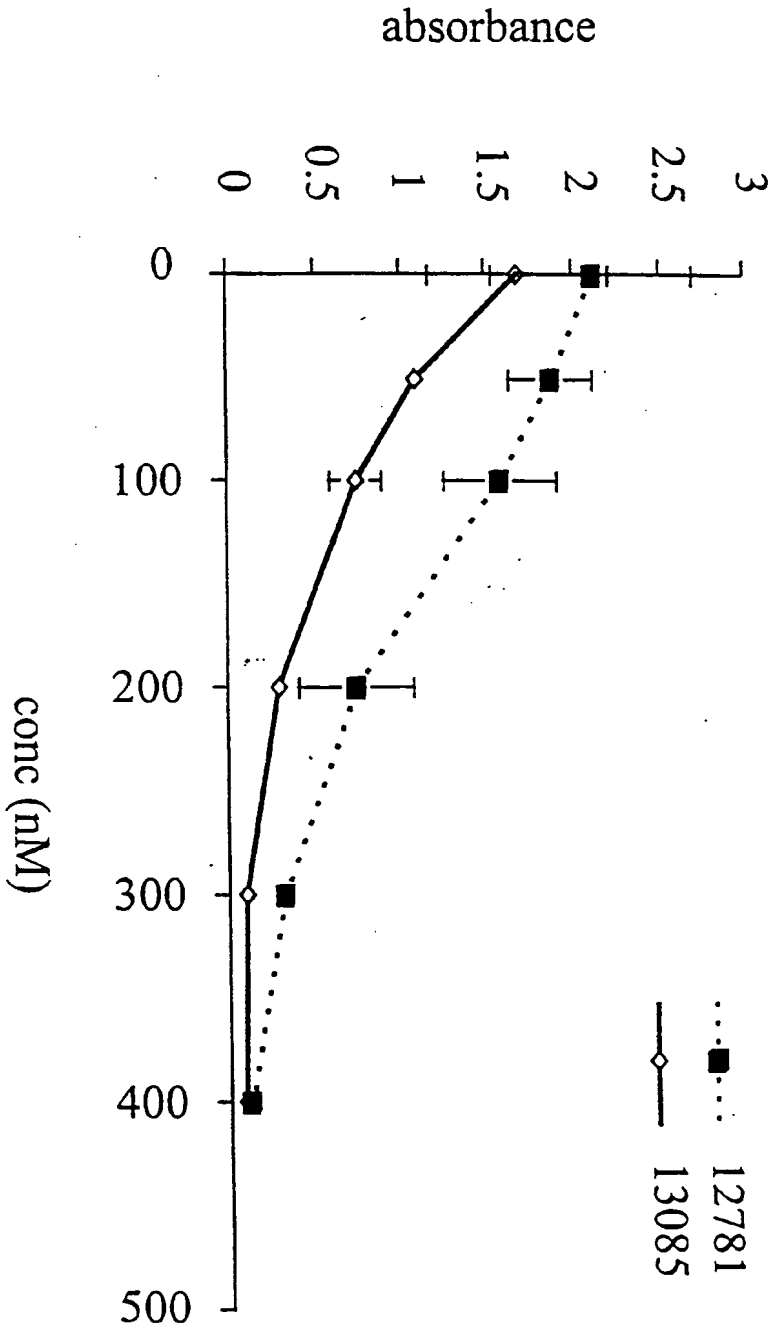
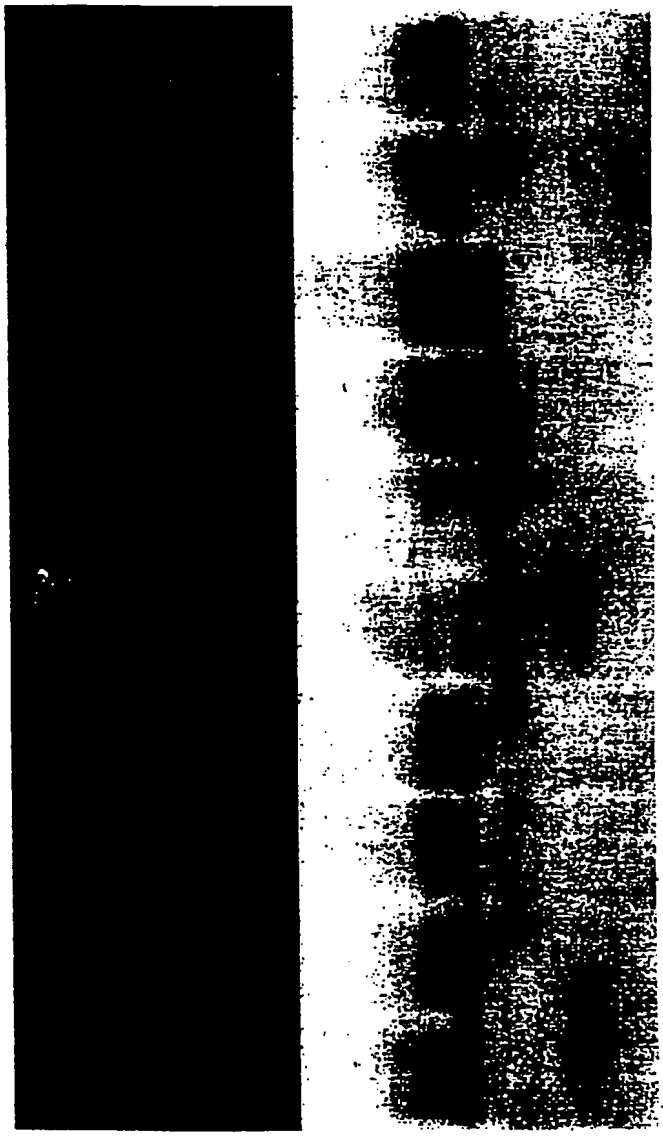

absorbance
0
0.5
1
1.5
2
2.5
3
conc (nM)
0
100
200
300
400
500
12781
13085

Fig. 16


confidential
Suppression of Ribonucleotide Reductase mRNA
RR1030 RR1031 RR2410ga RR2410ag 13704

MDA-MB 468 Cells

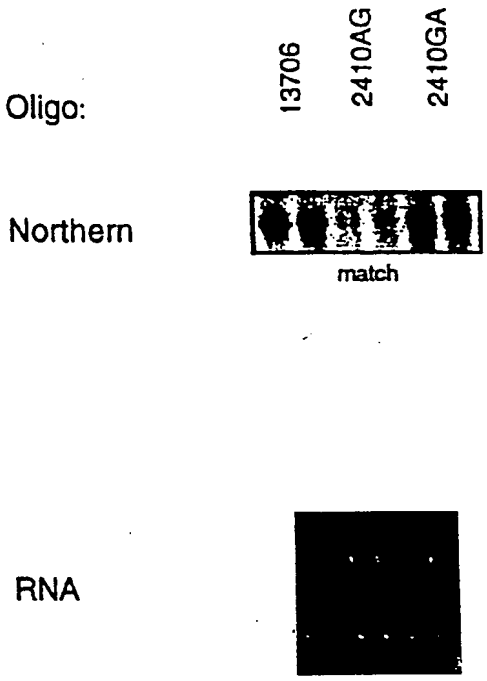


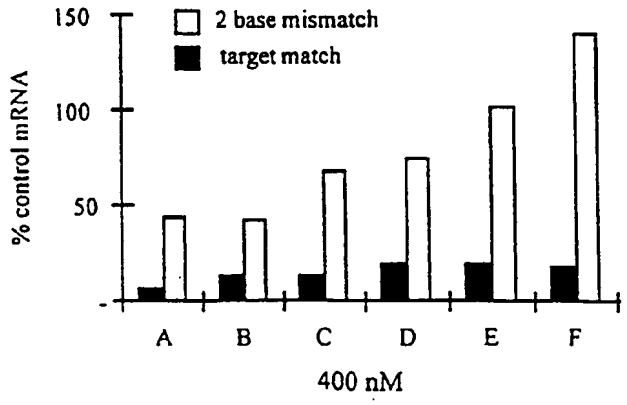
Fig. 17

Fig. 18

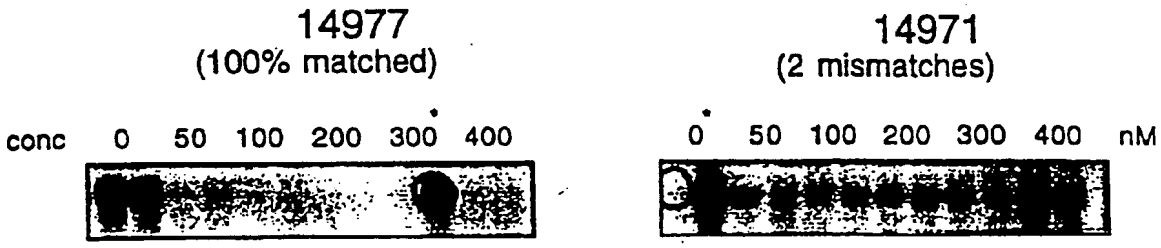
Research Collaboration

```
                    *       *
A     ACAGCCACTTATGTCATGGT
B     ACAGCCACTTATGTCATGGT
C     ACAGCCACTTATGTCATGGT
D          CACTTATGTCATGGTATTCA
E          CACTTATGTCATGGTATTCA
F          CACTTATGTCATGGTATTCA
```

Improved Allele-Specificity with Advanced Chemistry



Effect of Antisense Oligomers on Glutamyl-prolyl-tRNA Synthetase (EPRS) mRNA levels (duplicates)



*circled samples were switched when loaded on to the gel

Fig. 19